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ORIGINAL RESEARCH

Population-Specific Distribution of *TPMT* Deficiency Variants and Ancestry Proportions in Ecuadorian Ethnic Groups: Towards Personalized Medicine

Jennifer Gallardo-Cóndor[1], Pablo Naranjo[1], Sebastián Atarihuana[1], Dayana Coello[2], Patricia Guevara-Ramírez[3], Rodrigo Flores-Espinoza[4], Germán Burgos[5,6], Andrés López-Cortés[7,8], Alejandro Cabrera-Andrade[9,10]

[1]Facultad de Ingeniería y Ciencias Aplicadas, Universidad de Las Américas, Quito, Ecuador; [2]Laboratorios de Investigación, Universidad de Las Américas, Quito, Ecuador; [3]Centro de Investigación Genética y Genómica, Facultad de Ciencias de la Salud Eugenio Espejo, Universidad UTE, Quito, Ecuador; [4]Laboratório de Diagnóstico por DNA (LDD), Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil; [5]One Health Research Group, Facultad de Medicina, Universidad de Las Américas, Quito, Ecuador; [6]Grupo de Medicina Xenomica, Instituto de Ciencias Forenses, Universidad de Santiago de Compostela, Satiago de Compostela, Spain; [7]Cancer Research Group (CRG), Faculty of Medicine, Universidad de Las Américas, Quito, Ecuador; [8]Latin American Network for the Implementation and Validation of Clinical Pharmacogenomics Guidelines (RELIVAF-CYTED), Madrid, Spain; [9]Escuela de Enfermería, Facultad de Ciencias de la Salud, Universidad de Las Américas, Quito, Ecuador; [10]Grupo de Bio-Quimioinformática, Universidad de Las Américas, Quito, Ecuador

Correspondence: Alejandro Cabrera-Andrade, Grupo de Bio-Quimioinformática, Universidad de las Américas, UDLAPark 2, redondel del ciclista s/n, Quito, 170124, Ecuador, Tel +593 23 981 000 Ext.:2717, Email raul.cabrera@udla.edu.ec

Purpose: Thiopurine S-methyltransferase (TPMT) is an enzyme that metabolizes purine analogs, agents used in the treatment of acute lymphoblastic leukemia. Improper drug metabolism leads to toxicity in chemotherapy patients and reduces treatment effectiveness. *TPMT* variants associated with reduced enzymatic activity vary across populations. Therefore, studying these variants in heterogeneous populations, such as Ecuadorians, can help identify molecular causes of deficiency for this enzyme.

Methods: We sequenced the entire *TPMT* coding region in 550 Ecuadorian individuals from Afro-Ecuadorian, Indigenous, Mestizo, and Montubio ethnicities. Moreover, we conducted an ancestry analysis using 46 informative ancestry markers.

Results: We identified 8 single nucleotide variants in the coding region of *TPMT*. The most prevalent alleles were *TPMT*3A, TPMT*3B*, and *TPMT*3C*, with frequencies of 0.055, 0.012, and 0.015, respectively. Additionally, we found rare alleles *TPMT*4* and *TPMT*8* with frequencies of 0.005 and 0.003. Correlating the ancestry proportions with *TPMT*-deficient genotypes, we observed that the Native American ancestry proportion influenced the distribution of the *TPMT*1/TPMT*3A* genotype (OR = 5.977, p = 0.002), while the contribution of African ancestral populations was associated with the *TPMT*1/TPMT*3C* genotype (OR = 9.769, p = 0.003). The rates of TPMT-deficient genotypes observed in Mestizo (f = 0.121) and Indigenous (f = 0.273) groups provide evidence for the influence of Native American ancestry and the prevalence of the *TPMT*3A* allele. In contrast, although Afro-Ecuadorian groups demonstrate similar deficiency rates (f = 0.160), the genetic factors involved are associated with contributions from African ancestral populations, specifically the prevalent *TPMT*3C* allele.

Conclusion: The distribution of TPMT-deficient variants offers valuable insights into the populations under study, underscoring the necessity for genetic screening strategies to prevent thiopurine toxicity events among Latin American minority groups.

Keywords: thiopurine S-methyltransferase, TPMT deficiency, thiopurine toxicity, pharmacogenetics, ancestry estimation, Ecuadorian ethnic groups

Introduction

Thiopurine S-methyltransferase (TPMT) is a cytoplasmic enzyme that catalyzes the S-methylation of purine analogs, including azathioprine, 6-mercaptopurine (6-MP), and thioguanine.[1] The metabolism of these drugs results in two types of metabolites: S-methylmercaptopurine and S-methylthioguanine, which are generally described as inactive metabolites, and S-methyl-thioinosine monophosphate, an inhibitor of de novo purine synthesis. The cytotoxicity caused by these metabolites arises not only from the inhibition of purine synthesis but also from the alteration of intracellular signaling

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pathways and the incorporation of thioguanine nucleotides (TGNs) into DNA, thereby disrupting synthesis processes.[2] Given their ability to inhibit cell growth and promote cell death, these drugs are widely used as therapeutic agents, particularly in the treatment of acute lymphoblastic leukemia (ALL). They are also employed in the management of rheumatoid arthritis and immunological and inflammatory bowel diseases, as well as in post-transplant surgery.[3,4] However, excessive accumulation of TGN within cells can lead to severe cytotoxicity, necessitating careful monitoring and dosage adjustment to ensure optimal patient safety and treatment efficacy.[5]

Genetic polymorphisms play a critical role in modulating the enzymatic activity of TPMT, often resulting in an intermediate or complete reduction of activity. Early studies showed that TPMT deficiency is inherited as an autosomal codominant trait.[6] Following the discovery of *TPMT*2* (c.238C>G, rs1800462),[7] the characterization of *TPMT*3* alleles underscores the impact of *TPMT* variants on its enzymatic activity.[8] Specifically, the *TPMT*3* family encompasses the *TPMT*3B* (c.460G>A, rs1800460) and *TPMT*3C* (c.719A>G, rs1142345) alleles.[9,10] Moreover, the *TPMT3*A* allele arises from the combined presence of *TPMT*3B* and *TPMT*3C* variants.[11] The frequency of these variants is heterogeneous across different populations worldwide. While *TPMT*2* is primarily observed in European Caucasian populations,[12] *TPMT*3A* is predominantly distributed among Caucasian and Latin American populations. Conversely, *TPMT*3C* is common in East and South-East Asian and African populations.[13–17]

Genotype-phenotype association studies suggest that individuals who are heterozygous for one of these variants are predicted to be intermediate metabolizers (with approximately 50% enzyme activity), while individuals completely deficient in TPMT carry two distinct mutant alleles (referred to as compound heterozygotes) or are homozygotes.[18] Both intermediate-deficient and poor TPMT metabolizers have an increased risk of developing life-threatening myelosuppression upon receiving standard doses of thiopurine drugs.[19] They may also experience unexpected myelosuppression when they have been administrated the recommended doses of 6-MP. Given these findings, the Clinical Pharmacogenetics Implementation Consortium (CPIC) has provided guidelines and recommendations for dosing individuals carrying TPMT-deficient alleles. For intermediate metabolizers, especially those ALL patients treated with mercaptopurine, a 30–80% reduction in the starting dose of thiopurine medications is advised. For compound heterozygotes and homozygotes, a 10-fold dose reduction is recommended to avoid compromising the relapse rate.[20]

Toxicity in patients undergoing purine analog therapy does not solely arise from decreased TPMT enzymatic activity. Diminished activity of nucleoside diphosphate metabolizing enzymes (NUDT15) and inosine triphosphate pyrophosphatase (ITPA) significantly impacts the clinical presentation of adverse effects.[21,22] NUDT15 plays a pivotal role in the conversion of toxic thioguanine triphosphate (TGTP) metabolites into less harmful thioguanine monophosphate, and a reduction in its enzymatic activity results in elevated toxicity and cell apoptosis due to the failure to reduce TGTP concentrations in patients treated with 6-MP.[23] To date, 26 molecular variants associated with reduced or loss of function in *NUDT15* have been documented. However, specific variants, such as *NUDT15*2* (c. 55_56insGAGTCG and c.415C>T), *NUDT15*3* (c.415C>T), *NUDT15*7* (c.101G>C) and *NUDT15*9* (c.50delGAGTCG) are recognized as loss-of-function variants and are considered important pharmacogenetic markers for predicting toxicity.[24] The clinical relevance of specific variants, such as *NUDT15*3*, becomes especially significant in Asian populations due to their high prevalence in these groups. In contrast, African or European populations exhibit rare occurrences of this variant.[25,26] Additionally, the ITPA enzyme plays an essential role in protecting cells from DNA damage caused by non-canonical nucleotides by catalyzing the hydrolysis of inosine triphosphate into inosine monophosphate.[27] Despite the identification of specific variants linked to a 25–60% reduction in ITPA activity, like rs1127354 and rs7270101, subsequent studies have provided inconsistent findings regarding their correlation with adverse effects associated with 6-MP.[28] Consequently, current evidence does not endorse the routine use of pharmacogenetic testing for ITPA in clinical settings.[29]

Characterizing genetic variants in Latin American populations associated with drug response is vital for identifying groups at risk of potential toxicity when given specific medications. This knowledge becomes particularly valuable when translating clinical data from foreign populations to local ones.[30,31] Although the *TPMT*3A* allele is known to be prevalent in Latin American populations, research specifically addressing the distribution of defective *TPMT* alleles in the Ecuadorian population is lacking. Given the observed genetic diversity among Ecuadorian ethnic groups,[32] the need for such studies becomes even more evident. Therefore, our objective was to comprehensively identify *TPMT*-deficient

variants in the Afro-Ecuadorian, Mestizo, Indigenous, and Montubio groups by covering the entire coding region of the *TPMT* gene. The identification of pharmacogenetic variants within this gene will aid in implementing precise therapeutic strategies for patients undergoing thiopurine treatment and enable accurate genetic profiling for individuals in this Latin American population.

Materials and Methods

Sample Collection and DNA Extraction

A total of 550 blood samples from unrelated volunteers (274 male and 376 female individuals) aged 18 years and over were collected in EDTA tubes for sample preservation. All volunteers provided written informed consent prior to participation, which was approved by the Ethical Committee in Human Research at Universidad de las Américas (CEISH-UDLA 2017-0310). The study spanned three regions of Ecuador: the Coast (CO), Highlands (HG), and Amazon (AZ). Participants were classified according to their self-reported ethnicity into one of four groups: Afro-Ecuadorian (AFE), Indigenous (IND), Mestizo (MEZ), and Montubio (MON).

Our study focused on MEZ individuals from the CO (provinces of El Oro, Esmeraldas, Guayas, Los Ríos, Manabí, Santa Elena, and Santo Domingo), HG (Azuay, Bolívar, Carchi, Cañar, Chimborazo, Cotopaxi, Imbabura, Loja, Pichincha, and Tungurahua), and AZ (Morona Santiago, Napo, Pastaza, and Sucumbíos). MON participants reside in the provinces of Los Ríos and Manabí in the CO, whereas IND groups inhabit the HG (Chimborazo, Cotopaxi, and Imbabura), AZ (Morona Santiago, Napo, Pastaza, Orellana, and Zamora Chinchipe) and CO (province of Santo Domingo). Finally, AFE individuals are from the CO (province of Esmeraldas) and HG (provinces of Imbabura and Carchi).

DNA Isolation

Genomic DNA was extracted from blood samples using the salting-out method.[33] The genomic material was eluted in TE buffer in a final volume of 50 µL. DNA concentration was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) at 260 nm. A minimum concentration of 30 ng/µL and an absorbance ratio of 260/280 between 1.8 and 2 was set as the optimal range for subsequent procedures.

TPMT Genotyping

Genetic screening of the entire coding sequence (CDS) of *TPMT* was conducted using Sanger sequencing. To comprehensively examine genetic variation within this region, exons 3 to 10 were analyzed, and primers were positioned in the adjacent intronic regions. Primer design was carried out using the Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/, accessed on October 10th, 2022) and was based on the reference sequence NC_000006.12 (GRCh38.p14).

Polymerase chain reaction (PCR) was conducted in a final volume of 15µL, using GoTaq® Green Master Mix (Promega), following the manufacturer´s instructions. We used ~50 ng of DNA, 2x GoTaq Green Master Mix, and 0.9µM of each primer. An annealing temperature between 58 and 60°C was considered while 36 amplification cycles were performed for each primer. Post amplification, the PCR products were assessed via electrophoresis on a 2% agarose gel using a 100pb ThermoFisher® ladder.

Following this, the PCR products were purified by adding 0.5µL of alkaline phosphatase and 0.5µL of exonuclease to remove any remaining Master Mix and nucleotide residues. After purification, we amplified the amplicon using the BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems). The sequence products were then purified with Sephadex and run on the Genetic Analyzer 3130 (Applied Biosystems). Finally, data were analyzed using Sequencing Analysis v5.2 and SeqScape v2.5 (Applied Biosystems). The alignment with the reference sequence NC_000006.12 and the determination of variants in the coding region of *TPMT* was performed in the Geneious prime software (ver. 2022.1.1).

Analysis of Ancestry Proportions of Ancestral Populations

We analyzed the ancestry proportions of ancestral populations using 46 ancestry informative markers (AIMs). The genotyping was carried out through multiplex PCR tests, following the previously described reaction conditions.[34] The amplicons were separated using capillary electrophoresis in the 3130 Genetic Analyzer (Applied Biosystems). The resulting data were

analyzed using the GeneMapper v5 software (Applied Biosystems) and the allelic nomenclature previously described was used for genotype assignment.

Statistical Analysis

Arlequin v.3.5.2.2.2.2 software was used to calculate allele and genotypic frequencies, Hardy-Weinberg equilibrium (HWE), pairwise genetic distances (F_{ST}) and pairwise linkage disequilibrium (LD). In the HWE analysis, we established significant statistical differences considering p-values < 0.05. On the other hand, in LD analysis we adjusted the significance level by applying the Bonferroni correction. For each haplotype, we used Haploview 4.2 software to estimate the normalized linkage disequilibrium (D').

The inference of ancestry in the ethnic groups studied was derived from the admixture model with K = 3, based on the trihybrid composition described for the Ecuadorian population. This model was applied in STRUCTURE software v.2.3.4,[35] using runs comprising 100,000 burn-in steps followed by 100,000 Markov chain Monte Carlo (MCMC) runs without incorporating any a priori group information. We considered the genetic background of ancestral continental populations using African (AFR), and European (EUR), and Native American (NAM) reference samples obtained from the Human Genome Diversity Project at the Centre d'Etude du Polymorphisme Humain (HGDP-CEPH) diversity panel.

To assess the correlation between *TPMT* genotypes and ancestry proportions, we applied a Binomial Logistic Regression analysis. Results with p-values < 0.01 were deemed highly significant. This analysis was performed using IBM SPSS Statistics 22 software (SPSS Inc., Chicago, IL, USA).

Results

Genotyping of TPMT

We developed 8 primer sets for the amplification of the complete CDS of *TPMT*. The sequences of these primers, their positions in the reference sequence NC_000006.12, and the PCR conditions are described in Table S1. As shown in Figure 1, we recorded 34 single nucleotide variants (SNVs) distributed in the amplified regions of *TPMT*. Of these, 8 SNVs are in the CDS and 26 in the intronic region.

The minor allele frequencies (MAFs) observed for the nonsynonymous substitutions *TPMT*3C* (c.719A>G, rs1142345), *TPMT*3B* (c.460G>A, rs1800460), *TPMT*24* (c.537G>T, rs6921269), *TPMT*8* (c.644G>A, rs56161402), and c.122 A>G (rs757081801) were 0.075, 0.067, 0.005, 0.003, and 0.002, respectively. Moreover, the MAF for the synonymous substitutions was 0.788 for c.474C>T (rs2842934), 0.006 for c.339C>T (rs17839843) and 0.003 for c.579A>G (rs1783991183). We found no variation in *TPMT*2* (c.238G>C, rs1800462) within the population studied. The description of the allelic and phenotypic frequencies calculated for all the variants found are described in Table S2.

HWE, LD and Genetic Divergence

Statistically significant deviations from the HWE were detected in 9 of the 34 SNVs. Deviations from HWE were detected for loci c.122A>G (rs757081801), g.18147729T>C (rs17839846), c.233+96G>T (rs17839845), c.339C>T (rs17839843), g.18139571A>T (rs12201199), c.580+14G>T (rs2842949), c.626–257A>G (rs971032284), g.18130813T>C (rs143550474) and *TPMT*3C* (c.719A>G, rs1142345) in the ECU population. Additionally, 14 of these SNVs showed an excess of homozygotes compared with the expected in the HWE (Table S3). The average value of observed heterozygotes in the sample (0.099) was lower than that expected for a population in HWE (0.104). Since an excess of homozygotes suggests a genetic substructure and the frequency of these SNVs varies depending on the population, we repeated the analysis considering the 4 ethnic groups studied.

No significant deviations from the HWE were observed in the MON ethnic group. In contrast, significant deviations were observed in the AFE groups, particularly at loci c.233+96G>T (rs17839845) and c.626–257A>G (rs971032284). In the IND group, significant deviations were identified at loci c.122A>G (rs757081801), c.233+96G>T (rs17839845), and c.580+14G>T (rs2842949). It's noteworthy that, when considering the geographic distribution within the IND, the statistical significance disappeared for the locus c.122A>G (rs757081801). The MEZ group exhibited deviations at five analyzed loci, specifically at c.233+96G>T (rs17839845), c.626–257A>G (rs971032284), c.339C>T (rs17839843),

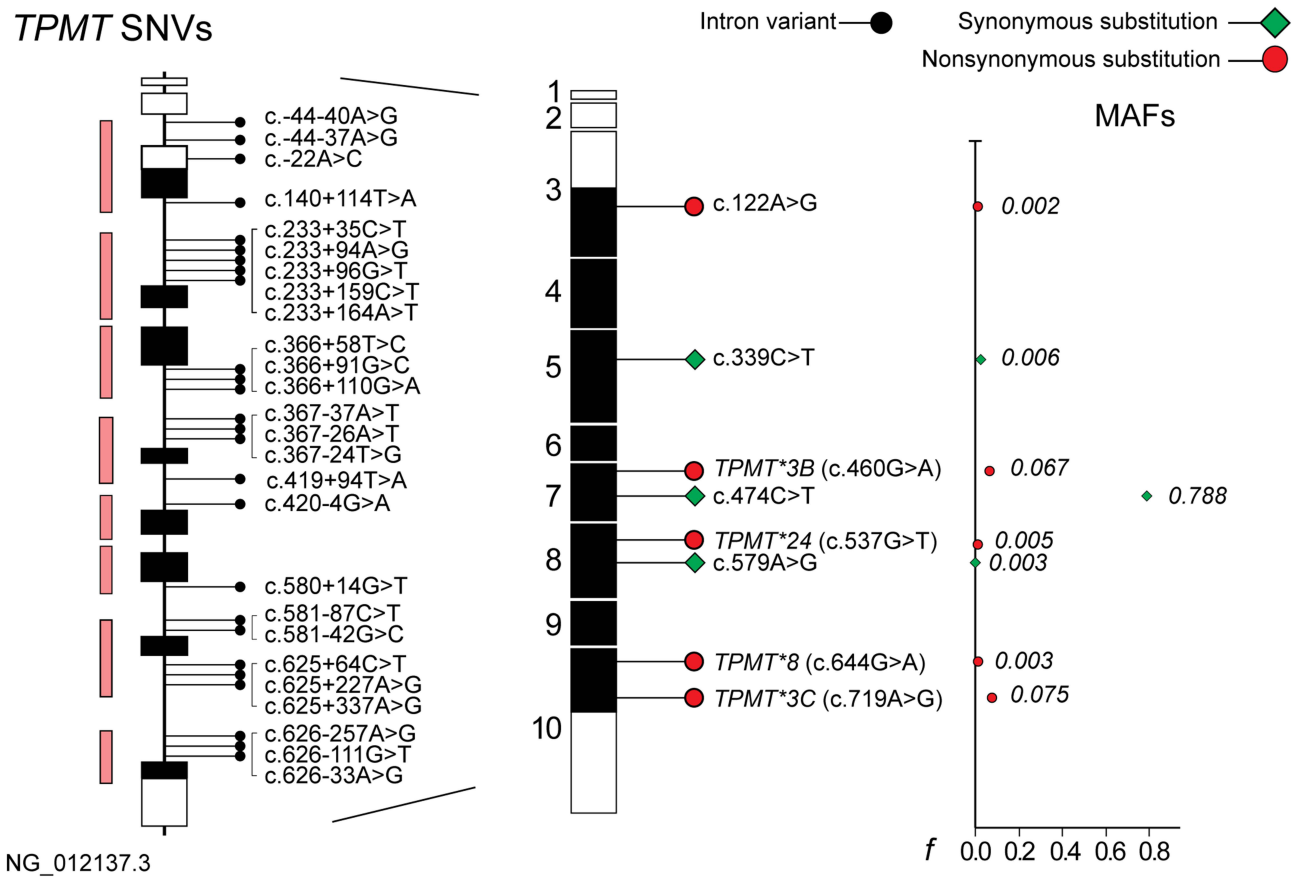

Figure 1 Schematic representation of the 8 amplified regions and the genetic variants found in the *TPMT* gene. The schematic displays the exonic regions (black and white boxes) and intronic regions of the *TPMT* gene. Intronic variants are indicated in the schematic to the left of the figure, while variants in the coding region are indicated in the central schematic. The minor allele frequencies (MAFs) of coding region variants are presented in the scatter graph.

g.18130813T>C (rs143550474), and *TPMT*3C* (c.719A>G, rs1142345). Notably, for all the variants under analysis, the observed mean number of heterozygotes was lower than expected for a population in HWE.

The significance values obtained from the HWE analysis point towards a potential population substructure, primarily attributed to the higher-than-expected number of homozygotes within the study groups. To validate this hypothesis, the degree of genetic divergence between the ethnic groups studied was assessed. The F_{ST} index indicates a genetic divergence between the AFE population and each of the following populations: IND (F_{ST} = 0.060, $p < 0.001$), MEZ (F_{ST} = 0.068, $p < 0.001$), and MON (F_{ST} = 0.08, $p < 0.001$). Furthermore, we observed genetic divergence between the IND groups and others, particularly with MEZ (F_{ST} = 0.006, p = 0.009) and MON (F_{ST} = 0.012, p = 0.018) populations (Table S4).

Pairwise linkage analysis revealed statistically significant associations between multiple loci, as presented in Table S5. Notably, when examining these results in conjunction with deficient *TPMT* variants, we observed that *TPMT*3A* showed a statistical association with *TPMT*3B* ($p < 0.001$). To explore whether the observed level of LD between these two variants might be attributed to ethnic variations, we conducted the analysis considering the 4 groups separately. By assessing the LOD values within these ethnic subsets and standardizing the gametic determinant D as D', we found that the *TPMT*3C* allele exhibited associations with six loci, including *TPMT*3B* (D' = 0.792, $p < 0.001$), c.419 +94T>A (D' = 0.810, $p < 0.001$), and c.140+114T>A (rs3931660) (D' = 0.793, $p < 0.001$). Similarly, a statistically significant association of *TPMT*3B* with c.140+114T>A (rs3931660) (D' = 0.862, $p < 0.001$), along with four additional loci, was observed (Figure 2). These findings suggest the formation of an LD block between these variants. Consequently, we

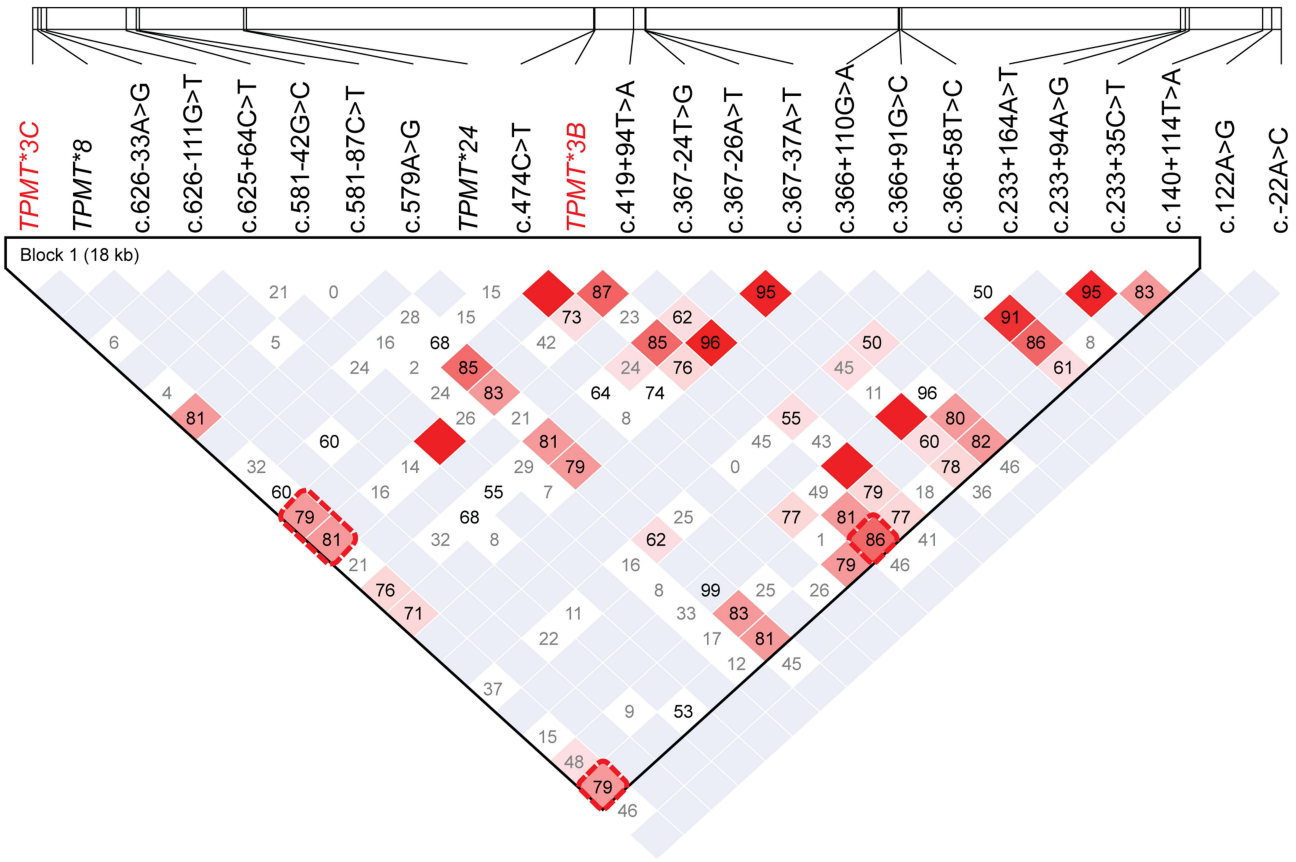

Figure 2 Block diagram representing the haplotypes of the 18 kb TPMT region investigated in this study. The analyzed SNVs and their positions within *TPMT* are denoted at the top of the figure (white bar). Pairwise LD between SNVs was examined using the standard D' coefficient (numbers in rhombi). The LD color scheme corresponds to the standard index (D'/LOD).

assume that *TPMT*3B* and *TPMT*3C* define the *TPMT*3A* haplotype within the studied groups. Subsequent analyses within the studied population take this association into account.

Distribution of TPMT Deficiency Genotypes Across Ethnic Groups

The TPMT enzymatic deficiency was determined by assessing the presence of *TPMT*3B* and *TPMT*3C* alleles in genotyped individuals. Accordingly, we classified individuals as *TPMT*1* when the c.460G>A/ c.719A>G diplotype was GA, as *TPMT*3B* when AA, and as *TPMT*3C* when GG. Based on the LD observed between *TPMT*3B* and *TPMT*3C*, we attributed the *TPMT*3A* allele to all individuals carrying both variants. The frequencies of imputed alleles and genotypes are detailed in Table 1.

It was found that 10.2% of the Ecuadorian population presents the *TPMT*1/TPMT*3A* genotype, 2.4% the *TPMT*1/TPMT*3B* genotype, 2.9% the *TPMT*1/TPMT*3C* genotype and 0.4% the *TPMT*3A/TPMT*3A* genotype. Considering the pooled frequency of these alleles with predictive value, 15.8% of the total population was identified as having intermediate and low enzyme activity.

The distribution of frequencies for deficient *TPMT* genotypes differs between the ethnic groups tested. While *TPMT*1/TPMT*3C* is more prevalent in AFE (n = 8, f = 0.08) individuals, *TPMT*1/TPMT*3A* occurs mostly in MEZ (n = 25, f = 0.086) and IND groups (n = 24, f = 0.188). Based on these predictive genotypes, 27.3% of IND groups present deficiency for *TPMT*, followed by AFE (16%), MEZ (12.1%), and MON (3.1%) groups.

Table 1 Distribution of *TPMT*3A, TPMT*3B, TPMT*3C*, and *TPMT*2* Alleles Across Different Ecuadorian Ethnic Groups and World Populations

Region	Country	n	*TPMT*3A*	*TPMT*3B*	*TPMT*3C*	*TPMT*2*	References
Central and South America	Ecuador	550	0.055	0.012	0.015	0.000	This study
	AFE	*100*	0.030	0.010	0.040	0.000	This study
	IND	*128*	0.102	0.023	0.016	0.000	This study
	MEZ	*290*	0.047	0.009	0.007	0.000	This study
	MON	*32*	0.160	0.000	0.000	0.000	This study
	Argentina	147	0.031	0.000	0.002	0.007	[36]
	Colombia	140	0.036	0.000	0.000	0.004	[13]
	Brazil	204	0.015	0.002	0.010	0.022	[37]
	Bolivia	115	0.065	0.000	0.000	0.000	[38]
	Guatemala	162	0.056	0.000	0.006	0.009	[17]
North America	USA (Caucasian)	282	0.032	0.000	0.002	0.002	[39]
	USA (African-descendent)	248	0.008	0.000	0.024	0.004	[39]
	Mexico	108	0.032	0.023	0.014	0.009	[40]
Europe	British	199	0.045	0.000	0.003	0.005	[15]
	Sweden	800	0.038	0.001	0.004	0.001	[41]
	Germany	1214	0.044	0.000	0.004	0.002	[42]
	Italy	103	0.039	0.000	0.009	0.004	[43]
	Poland	358	0.027	0.000	0.001	0.004	[44]
	Bulgarian	313	0.022	0.000	0.002	0.002	[45]
	Norway	66	0.034	0.000	0.003	-	[46]
Asia	Chinese	225	0.000	0.000	0.013	0.000	[47]
	India	200	0.000	0.000	0.023	-	[48]
	Tibet	50	0.000	0.000	0.010	0.000	[38]
	Thailand	200	0.000	0.000	0.050	0.000	[49]
	Japanese	192	0.000	-	0.008	0.000	[50]
Middle-East	Turkish	106	0.009	0.000	0.009	0.009	[51]
	Iran	832	0.017	0.016	0.005	0.022	[52]
Africa	Ghana	217	0.000	0.000	0.076	0.000	[15]
	Kenya	101	0.000	0.000	0.054	0.000	[53]
	Egypt	200	0.003	0.000	0.013	0.000	[54]
	Mozanbique	250	0.002	0.000	0.038	0.000	[55]

Notes: The number of individuals (n) described in each study and the reported frequencies for the *TPMT*3A, TPMT*3B, TPMT*3C* and *TPMT*2* alleles are presented. The *TPMT*3A* frequencies reported in this study are derived from the allelic frequencies of *TPMT*3B* and *TPMT*3C* and are shown for total population (ECU), Afro-Ecuadorian (AFE), Indigenous (IND), Mestizo (MEZ), and Montubio (MON).

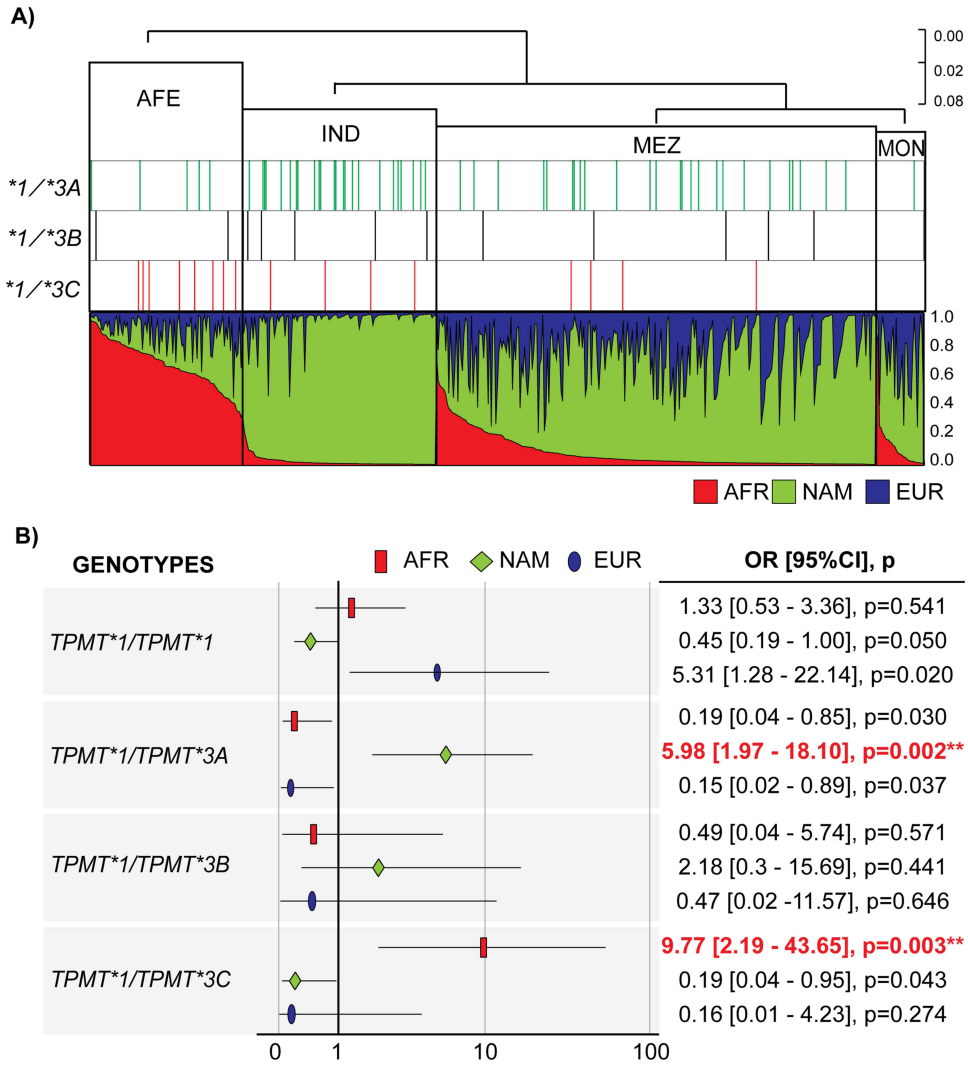

Figure 3 Ancestry proportions in Ecuadorian population and *TPMT* deficient genotypes. (**A**) Distribution of individuals with *TPMT*1/TPMT*3A, TPMT*1/TPMT*3B*, and *TPMT*1/TPMT*3C* genotypes and their ancestry proportions calculated for the African (AFR), European (EUR) and Native America (NAM) reference populations (k = 3, assuming migration model). The dendrogram clusters the 4 ethnic groups according to the F_{ST} values calculated from SNVs found in the *TPMT* gene. (**B**) Forest plot showing odds ratios (OR) for *TPMT*-deficient genotypes and the contribution of ancestral AFR (red box), NAM (green diamond), and EUR (blue oval) populations. Error bars represent 95% confidence intervals (CI). Significance denoted with double asterisk at p-values <0.01.

Correlation Between Ancestry Proportions and TPMT Deficiency Genotypes

As seen in Figure 3A, we integrated the distribution of *TPMT*-deficient genotypes across ethnic groups with the calculated proportions of ancestry. The cluster analysis based on all *TPMT* SNVs reveals differences between AFE and IND groups, and particularly between MEZ and MON. This clustering not only reflects the differential distribution of *TPMT* variants in the studied population but also describes the differences found with the contributions of ancestral populations for each group.

The overall majority contribution to the Ecuadorian population comes from the NAM ancestral population ($f = 0.641$), followed by AFR ($f = 0.182$), and EUR ($f = 0.177$). Upon examining the distribution within ethnic groups, there is a majority contribution of the NAM populations for the IND ($f = 0.924$), MEZ ($f = 0.674$), and MON ($f = 0.550$) groups. In contrast, the AFE groups exhibit a higher proportion of AFR ancestral populations ($f = 0.663$). Interestingly, the calculated EUR contribution aligns with the described admixture history for the MEZ and MON groups ($f = 0.246$ and 0.312, respectively), while this proportion decreases in the AFE ($f = 0.125$) and IND ($f = 0.050$) groups.

Statistically significant associations were observed when examining the relationship between the calculated ancestry proportions and *TPMT* genotypes. As shown in Figure 3B, the contribution of NAM ancestry populations is positively correlated with the *TPMT*1/TPMT*3A* genotype within the total population, with an odds ratio (OR) of 5.977 (CI = 95%: 1.967–18.143, p = 0.002). Conversely, AFR ancestry contribution is associated with the *TPMT*1/TPMT*3C* genotypes, demonstrating an OR = 9.769 (CI = 95%: 2.187–43.645).

Discussion

Our screening strategy led to the detection of 26 SNVs in the intronic region and 8 variants in the entire screened CDS. Out of these, 5 were nonsynonymous substitutions and 3 were synonymous substitutions. While approximately 10,300 SNVs are described for *TPMT*, most of these variants are in non-coding regions (83.55% in the intronic region and 11.5% in the 5' and 3' untranslated regions). Given that only 3.06% of the variants described for *TPMT* occur in the CDS (2.07% nonsynonymous and 0.69% synonymous),[56] the low rate of SNVs in the CDS found in the studied population aligns with the molecular variability reported for this gene.

The observed genetic diversity among the 34 SNVs identified in the screened regions of *TPMT* across various ethnic groups reflects their distinct evolutionary histories, influenced by variations in migration patterns, historical population sizes, and selective pressures. In the total population, the HWE analysis reveals deviations for 9 loci. However, when we consider the ethnic groups defined within the study, the deviations are reduced to 4 loci in the MEZ group, 2 in AFE, and 2 in IND (as detailed in the results section). The overrepresentation of homozygotes in this analysis indicates a substructure within the population, aligning with previous descriptions of the complex genetic makeup of the Ecuadorian population.[57] Within the studied ethnic groups, the MEZ population exhibits a intricate genetic structure resulting from varying admixture patterns involving European, African, and Native American populations.[58–61] These findings, coupled with the F_{ST} divergence analysis, substantiate the heterogeneity of the studied population and emphasize that the significant genetic variability observed among Ecuadorian ethnic groups not only underscores cultural distinctions but also underscores genetic diversity.[62] The *TPMT* variants we have described offer a comprehensive genetic characterization of the studied ethnic groups, particularly considering the allele distribution within the MEZ, IND, and AFE groups.

About 40 *TPMT* alleles (*TPMT*2* to *TPMT*41*) associated with a reduction in TPMT enzymatic activity have been described.[63,64] However, *TPMT*2* (c.238G>C, rs1800462), *TPMT*3B* (c.460G>A), *TPMT*3C* (c.719A>G), and *TPMT*3A* (c.460G>A and c.719A>G) collectively account for more than 95% of phenotypic TPMT variability.[65] Significant pairwise LD associations were detected between *TPMT*3B, TPMT*3C*, c.140+114T>A (rs3931660), and c.419+94T>A (rs12201199), both within the overall population and across specific ethnic groups. These associations, in line with prior studies,[42,66,67] serve a dual purpose. Firstly, they underscore the significance of c.140+114T>A and c.419 +94T>A as predictive markers in TPMT. Secondly, they enable us to infer the presence of the *TPMT*3A* allele in the studied population. Within our screened cohort, *TPMT*3A, TPMT*3C*, and *TPMT*3B* alleles emerge as the most prevalent pharmacogenetic markers, with frequencies of 0.055, 0.015, and 0.012, respectively. These variants, as reported previously,[36,40,68] are the primary genetic contributors to TPMT deficiency in Latin American groups. *TPMT*3A*, with frequencies ranging from 0.022 to 0.045 in European populations and up to 0.065 in Latin American groups, is the most common allele. As indicated in Table 1, the values observed in Ecuadorian groups (f = 0.055), especially in MEZ (f = 0.047), closely resemble those documented in Latin American populations, and especially with Bolivian groups where *TPMT*2* is absent. The distribution of deficient *TPMT* alleles demonstrates distinct ethnographic patterns across global populations. In Asian and African groups, *TPMT*3C* accounts for the genetic basis of deficiency, with frequencies ranging from 0.008 to 0.090 and 0.013 to 0.076, respectively (Table 1). It is important to note that AFE groups exhibit *TPMT*3C* frequencies (f = 0.040) similar to those in these populations, including North American African-descendant groups. These findings align with the migratory history and genetic composition documented for the AFE group.[69]

TPMT deficiency in the Ecuadorian population was analyzed based on the variability found in the *TPMT*3A, TPMT*3B*, and *TPMT*3C* alleles, excluding the loci c.122A>G (rs757081801), *TPMT*24* (c.537G>T, rs6921269), and *TPMT*8* (c.644G>A, rs56161402). Although these loci are associated with reduced TPMT enzymatic activity,[55,56] we did not consider the frequencies of these variants in the calculation of TPMT deficiency for the Ecuadorian population

due to their low prevalence, both in the general population and in this study. The most common genotype observed was *TPMT*1/TPMT*3A* ($f = 0.102$), followed by *TPMT*1/TPMT*3C* ($f = 0.029$) and *TPMT*1/TPMT*3B* ($f = 0.024$). Our study also identified only two individuals with the homozygous *TPMT*3A/TPMT*3A* genotype ($f = 0.004$). Based on these findings, the calculated deficiency of *TPMT* is 15.8%.

The *TPMT*3C* variant is suggested to be the first mutation associated with TPMT deficiency present in ancestral African populations, with the *TPMT*3B* mutation developing later. This second event likely occurred after the divergence of African and non-African populations, leading to the *TPMT*3A* allele (*TPMT*3B* and *TPMT*3C*).[70] To explain the distribution of deficient *TPMT* alleles, we considered not only the participants' self-reported ethnicity but also estimated the contributions of EUR, NAM, and AFR ancestral populations in the different groups. The main contribution within the Ecuadorian population is NAM ($f = 0.641$), followed by EUR ($f = 0.182$) and AFR ($f = 0.177$). Additionally, the proportion of NAM ancestral populations is the most prevalent in IND, MEZ, and MON groups. *TPMT*3A* is the most frequent allele in our population, accounting for 67.4% of the calculated TPMT deficiency in the total population, and approximately 75% in the MEZ and INF groups. Based on this distribution and by applying binomial logistic regression, we found a positive correlation between the ancestry proportions of MEZ populations and *TPMT*1/TPMT*3A* genotypes (OR = 5.977, CI = 1.967–18.143) (Figure 3B). This correlation suggests that individual proportions of NAM ancestry significantly influence the distribution of *TPMT*3A*, supporting the hypothesis suggested by previous authors.[71,72]

Moreover, we found that the contribution from ancestral AFR populations influences the presence of the *TPMT*3C* allele in the ECU population (OR = 9.769, CI = 2.187–43.645), particularly in AFE groups (Figure 3B). Fifty percent of *TPMT*-deficient individuals in AFE groups carry *TPMT*1/TPMT*3C* genotypes, while the remaining 50% carry *TPMT*1/TPMT*3A* and *TPMT*1/TPMT*3B* genotypes. Interestingly, the genetic background estimated by ancestry informative markers describes this distribution of deficient genotypes, not only for *TPMT*3C* but also for *TPMT*3A*. Although AFE groups demonstrate a significant contribution from AFR populations (0.663), their history of admixture with NAM groups from the 16th century onwards is evident when considering the NAM contribution (0.213) to this Afro-descendant group.[62] This finding highlights the impact of historical admixture on the genetic composition of AFE groups and the distribution of TPMT deficiency within this population.

Our study highlights the influence of the genetic contribution from ancestral populations on the distribution of deficient *TPMT* alleles, carrying significant implications for the adoption and interpretation of pharmacogenetic tests for thiopurine drugs across Latin American groups.[73] Thiopurines are a class of medicine for which the investigation of inherited genetic variations is considered substantial evidence to incorporate pharmacogenomics into clinical practice.[74] *TPMT* genotypes are crucial molecular biomarkers for predicting drug response in the treatment of hematological malignancies, autoimmune diseases, and organ transplants.[75–77] For instance, testing for *TPMT* genotypes at the beginning of maintenance therapy in patients with ALL can reduce treatment interruptions and the subsequent risk of relapse.[78,79] Patients with homozygous *TPMT* variants tolerate only 10% of recommended doses (2.5 mg/kg), while heterozygotes have up to four times the risk of neutropenia and require a dose reduction of 50%.[80] A skewed metabolism of 6-MP can cause gastrointestinal toxicity, including refractory nausea, pancreatitis, and hepatotoxicity, which can significantly limit the tolerable dose.[18,20,81–83] Given the calculated prevalence of TPMT deficiency in the Ecuadorian population and the heterogeneous distribution of genetic factors linked to this condition among the various ethnic groups under examination, it becomes paramount to institute genetic screening measures to mitigate the risk of severe adverse effects stemming from thiopurine toxicity. These screening strategies should encompass the assessment of *TPMT*3A, TPMT*3B* and *TPMT*3C* alleles, with a particular emphasis on their application within IND and AFE minority groups, where the probability of their occurrence is higher compared to the MEZ group.

This study provides a comprehensive overview of the prevalence and distribution of TPMT variants associated with reduced enzymatic activity across various Ecuadorian ethnic groups. This not only allows us to identify the genetic factors linked to thiopurine-induced toxicity but also sets the stage for the implementation of targeted genetic screening strategies in this population. However, it is important to note that further investigations are required to explore the presence of additional markers in *NUDT15*, which will contribute to a more comprehensive understanding of the genetic

underpinnings of toxicity in this population. One of the main limitations of our study is the imputation method employed in defining *TPMT*3A*. While Sanger sequencing proved to be a robust technique for evaluating the genetic variability of the CDS of *TPMT*, its inability to detect compound heterozygotes, such as *TPMT*3B/*3C*, may result in an elevated rate of false positives for *TPMT*3A*. It's worth mentioning that using sequencing as a screening approach allows us to accurately identify *TPMT*1/ TPMT *1* individuals with sensitivity, specificity, and predictive values exceeding 90%.[84] While the reported frequencies of this allele in our study may be subject to errors, it is crucial to emphasize that this method effectively narrows down the population where *TPMT*-deficient genotypes need to be determined. In the context of screening programs, more sensitive techniques like allele-specific (AS)-PCR, PCR-restriction fragment length polymorphism (PCR-RFLP), gel-based DNA-microchip technology, or denaturing high-performance liquid chromatography could be considered for improved detection sensitivity.

Conclusion

Genetic screening conducted on the Ecuadorian population has revealed the presence of seven single nucleotide variants within the entire coding region of *TPMT*. Among these variants, the most common pharmacogenetic alleles are *TPMT*3A* (0.055), *TPMT*3B* (0.012), and *TPMT*3C* (0.015), while the rare alleles *TPMT*4* and *TPMT*8* are observed with frequencies of 0.005 and 0.003, respectively. Based on the frequency of these variants, the calculated TPMT deficiency rate for the Ecuadorian population is 0.158.

The distribution of TPMT-deficient variants is closely linked to the genetic contribution of ancestral populations. In both the IND and MEZ groups, where the main ancestry proportion is NAM (0.924 and 0.674, respectively), the high prevalence of individuals with the *TPMT*1/TPMT*3A* genotype suggests an influence of this genetic background on the presence of this marker (OR = 5.977, p = 0.002). Conversely, in AFE populations, where the main contribution is from AFR ancestral populations (f = 0.663), the high prevalence of individuals with the *TPMT1/TPMT*3C* genotype aligns with their genetic background (OR = 9.769, p = 0.003). Our findings emphasize the influence of genetic inheritance from ancestral populations on the prevalence of *TPMT*-deficient alleles. The distribution patterns of pharmacogenetic variants on *TPMT* not only provide valuable insights into the genetic composition of these populations but also underscore the importance of implementing targeted genetic screening strategies. These findings have significant implications for the utilization and understanding of pharmacogenetic testing for thiopurine drugs in Latin America.

Abbreviations

6-MP, 6-mercaptopurine; AFE, Afro-Ecuadorian; AFR, African; AIMs, ancestry informative markers; ALL, acute lymphoblastic leukemia; AZ, Amazon; CDS, coding sequence; CO, Coast; EUR, European; HG, Highlands; HWE, Hardy-Weinberg equilibrium; IND, Indigenous; MAF, minor allele frequency; ITPA, inosine triphosphate pyrophosphatase; LD, linkage disequilibrium; MEZ, Mestizo; MON, Montubio; NAM, Native American; NUDT15, nucleoside diphosphate metabolizing enzymes; OR, odds ratio; SNVs, single nucleotide variants; TGNs, thioguanine nucleotides; TGTP, thioguanine triphosphate; TPMT, Thiopurine S-methyltransferase.

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Disclosure

The authors declare no competing interests in this work.

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