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Pharmacogenetic Study of Anti-TB Drugs in the Native Ancestry Peruvian Population

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ABSTRACT

In Peru, 33 113 individuals were diagnosed with tuberculosis (TB) in 2023. While TB treatments are generally effective, 3.4% to 13% of cases are associated with significant adverse drug reactions, with drug-induced liver injury (DILI) being the most prevalent. Limited data exist on genetic risk factors for DILI in Latin America; even less is known about these factors in native Peruvian populations. This study aimed to determine the prevalence of TB drug-metabolizing genotypes in these populations. A cross-sectional analysis was conducted using genetic data from 254 participants from the Peruvian Genome Project (PGP) representing three subpopulations: Coast, Andes, and Amazon. Twenty-three genes associated with TB treatment, include isoniazid, rifampin, ethambutol, and pyrazinamide, as identified in the PharmGKB database, were analyzed. Significant differences were observed in genotype frequencies among subpopulations for *AGBL4, NAT2, GSTP1, SLCO1B1, NOS*, and *CYP2B6* genes. The Amazonian population demonstrated a higher risk of DILI due to the increased prevalence of hepatotoxic alleles in *AGBL4, GSTP1*, and *SLCO1B1*. In contrast, alleles in the *NOS* gene indicated a lower risk of hepatotoxicity in the Andean population. However, the high-risk genotypes identified in the study's native Peruvian populations exhibit distinct prevalence patterns compared to those reported in the 1000 Genomes Project. These findings can inform the development of personalized therapeutic strategies to improve TB treatment outcomes among Peru's diverse subpopulations.

1 | Background

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* and is among the top 10 leading causes of death worldwide [1]. TB affects anyone at any age but has a greater impact on the working-age population [2]. In 2022, TB caused approximately 1.3 million deaths worldwide. It predominantly affects lower-income populations, though it can impact individuals across all socioeconomic levels. In Peru, 33 113 people were diagnosed with TB in 2023 [1].

In Peru, first-line drug treatment had a success rate of 87.2%, according to the World Health Organization (WHO) in 2016 [1]. Although TB treatments are effective, 3.4%–13% are

Abbreviations: *AGBL4*, ATP/GTP binding protein like 4; *CYP2B6*, cytochrome P450 family 2 subfamily B member 6; DILI, drug-induced liver injury; *GSTP1*, glutathione S-transferase Pi 1; INH, isoniazid; MDR-TB, multidrug-resistant tuberculosis; *NAT2*, *N*-acetyltransferase 2; *NOS*, nitric oxide synthase; PGP, Peruvian Genome Project; *SLCO1B1*, solute carrier organic anion transporter family member 1B1; TB, tuberculosis; WHO, World Health Organization.

associated with significant adverse drug reactions, with drug-induced liver injury (DILI) considered the most predominant [1]. DILI is a significant adverse reaction associated with anti-tuberculosis treatment, creating delays in therapy and increasing the likelihood of drug resistance. In the human liver, isoniazid (INH) is first acetylated by *N*-acetyltransferase 2 (NAT2) to acetylhydrazine, then oxidized into toxic intermediates by Cytochrome P450 family 2 subfamily E member 1 (CYP2E1) [3]. The toxic compounds produced are detoxified through acetylation by NAT2 and conjugation reactions catalyzed by Glutathione S-Transferase Pi 1 (GST) enzymes. Several risk factors for DILI have been identified, including co-infection with HIV, hepatitis B or C, advanced age, and female gender [4, 5].

Additionally, genetic variations can influence drug metabolism, leading to differences in antibiotic concentrations in the blood. For example, individuals with genetic traits associated with increased metabolism may process antibiotics too quickly, reducing their effectiveness, while slow metabolizers may have prolonged drug exposure, increasing the risk of toxicity [6, 7]. Genes such as *NAT2, CYP2E1* have been evaluated in different populations worldwide, including Americans, Africans, Europeans, and Asians [8–10]. A multinational study that included individuals from Peru reported that the *NAT2*4*, **1*, **12*, **13*, **18* genotypes were associated with intermediate and fast metabolizers. However, this study did not provide sufficient evidence to optimize the concentration of the drug and reduce the side effects of isoniazid in the Peruvian populations diagnosed with tuberculosis [11].

Studies of genetic diversity in populations are important because they identify the frequencies of polymorphisms for each population. The basis of pharmacogenomics is that while standard drug selection and dosage guidelines apply across populations, the distribution of metabolizer phenotypes (e.g., intermediate metabolizers [IMs], poor metabolizers [PMs]) varies. For example, genetic variations in *NAT*2 affect the metabolism of isoniazid, a first-line drug for tuberculosis. Populations with a higher prevalence of slow acetylators (NAT2 slow metabolizers) may experience increased drug toxicity, whereas fast acetylators may have reduced therapeutic efficacy. Genetic ancestry analysis of the Peruvian mestizo population, derived from multiple Native American communities, has revealed distinct genetic profiles [12]. These differences highlight the limitations of extrapolating pharmacogenomic data from other populations. For example, variations in *NAT2* alleles, which influence isoniazid metabolism in tuberculosis treatment, show different frequencies in Peruvian populations compared to others, affecting drug response and the risk of adverse effects [11].

Thus, in 2018, the technical report by WHO experts underscores an important role in determining metabolization phenotypes during drug administration. For example, for the administration of isoniazid, in children and adults, the dose is defined as 10–15 mg/kg/day in multidrug-resistant tuberculosis (MDR-TB) regimens (the usual dose is 4–6 mg/kg/day). However, it notes that in North Asia, where most of the population has the rapid metabolizer phenotype, a dose of 15 mg/kg may be more effective [13].

Several studies have reported that pharmacogenetic variations in *NAT2* influence pharmacokinetics and contribute to differences in toxicity following isoniazid treatment [14–16]. A previous study concludes that patients with a fast metabolizer should receive 50% more than the standard dose, while patients with a slow genotype should receive half the standard dose [17]. Azuma et al. reported that 78% of the slow metabolizers experienced hepatotoxicity due to being treated with standard doses of INH, while none of those who received the modified dose reported liver damage [6]. According to the above, the benefit of the modified treatment in reducing DILI according to the metabolization of INH was demonstrated [18].

This study highlights the unmet need to identify individuals at genetic risk for DILI, enabling the development of personalized therapeutic regimens that mitigate these risks. By targeting high-risk genotypes, alternative treatments can be offered, reducing the burden of DILI and improving treatment adherence and outcomes in affected populations. Although the main mechanisms involved in developing hepatotoxicity are known, this field has not been significantly explored in Peru, particularly in native ancestry populations. This study aims to estimate the prevalence of metabolizing genotypes in patients during tuberculosis treatment in native Peruvian populations.

2 | Patients and Methods

2.1 | Studied Population and Participants

This research is a secondary analysis of data from a broader cross-sectional study conducted by the Peruvian NIH. The primary study aimed to investigate genetic diversity in populations from Peru, reporting clinically relevant single nucleotide polymorphisms (SNPs) among Latin American groups [12]. This secondary study investigate genetic diversity and pharmacogenetic variations in native Peruvian populations. By utilizing existing genetic datasets, this study aims to identify population-specific metabolic profiles that influence TB drug response, thus informing more effective, personalized treatment strategies. A total of 254 samples were analyzed. Inclusion criteria consisted of individuals from PGP with more than 95% native ancestry based on bioinformatics analysis results (Coast, Andes, Amazon), and informed consent for genetic testing. Exclusion criteria included individuals with incomplete genetic data.

2.2 | Consent

The study was approved by the Ethics in Research Committee of the Peruvian National Institute of Health (authorizations OI-003-11 and OI-087-13) and was conducted in accordance with the principles of the Declaration of Helsinki. Informed written consent was obtained from all participants.

2.3 | Genetic Data and Quality Control

Peripheral blood samples (4 mL) were collected from participants, and DNA was extracted using the QIAamp DNA Blood

Mini Kit (Qiagen, CA, USA). DNA samples were genotyped using the Illumina Omni 2.5M array in three batches at the Peruvian NIH facilities.

One batch, consisting of 50 individuals, was genotyped using GRch38. This batch was lifted to GRch37 using the UCSC liftover tool (https://genome.ucsc.edu/cgi-bin/hgLiftOver) before merging with other GRch37 batches. After merging, we removed SNPs and individuals with more than 5% missing genotypes using PLINK [19]. Variants with AT/CG genotypes or those out of Hardy–Weinberg equilibrium (HWE) ($p < 10e^{-5}$) were excluded. Related individuals (first-degree relatives) were removed (king-cutoff 0.177) using PLINK. Array data was imputed against the TOPMed imputation panel (https://imputation.biodatacatalyst.nhlbi.nih.gov/#!). Briefly, the TOPMed imputation server performs the phasing of the query data using Eagle2 and imputation using minimac4.

2.4 | Admixture Analysis

We used ADMIXTURE [20] to explore genome-wide ancestry proportions. In this analysis, K represents the number of ancestral populations assumed in the model. We tested values of K ranging from 4 to 8 in a subset of individuals that includes all samples (admixed and Native Americans), a subgroup of 1000 Genomes Project (1KGP) high coverage populations with unrelated individuals (TSI, IBS, CEU, PEL, CLM, MXL, PUR, LWK, MSL, YRI, GWD, JPT, CHB, and CHS), and Native Americans from the Human Genome Diversity Project (HGDP) (Colombian, Pima, and Maya populations). This subset included unlinked single nucleotide variants and a minor allele frequency (MAF) > 5%. We performed ten runs per *K* value and plotted the run with the highest log-likelihood.

We performed local ancestry by running RFMIXver2 [21–23] with reference panels from European, African, East Asian, and Native American populations. Individuals with > 95% Native American ancestry were classified into Andean, Coastal, or Amazonian groups based on recruitment location. We selected European ($n = 404$ individuals; populations included CEU, GBR, IBS, and TSI), African ($n = 405$ individuals; populations included YRI, ESN, GWD, and LWK), and East Asian ($n = 411$ individuals; populations included CHB, CHS, JPT, and KHV) individuals from 1KGP populations. Native American reference included 187 individuals from PGP with more than 99% NAT (Native American ancestry) based on ADMIXTURE K4 results. We ran RFMIX using two expectation maximization steps.

2.5 | Measures and Analysis

For this analysis, we only included individuals with > 95% NAT ancestry, resulting in 254 non-admixed participants. Individuals were classified into Coast, Andes, or Amazon native subpopulations depending on the location of their recruitment site. We explored the relationship between native subpopulations and 23 genetic markers associated with TB treatment identified in the PharmGKB database (https://www.pharmgkb.org/) (Table S1). A high-risk genotype is defined as a genetic variant or combination of variants associated with an increased likelihood of adverse drug reactions, particularly DILI, in the context of TB treatment. These genotypes are identified based on prior pharmacogenetic research and databases, such as PharmGKB, and are evaluated for their prevalence and association with hepatotoxicity. Genotypes not linked to elevated DILI risk or associated with protective metabolic profiles were categorized as low-risk. Statistical analysis, including chi-square tests and regression models, determines significant differences in the frequency of these high-risk genotypes across the studied subpopulations. To account for multiple testing, a Bonferroni correction was applied, adjusting the significance threshold by the number of independent tests performed. Statistical significance was set at $p < 0.05$ using Stata 15 (StataCorp, College Station, TX, USA).

3 | Results

A total of 27 SNPs associated with TB drug metabolism were analyzed, as reported in the PharmGKB database (Table S1). These SNPs span key pharmacogenetic genes such as *NAT2, CYP2E1, GSTP1*, and Solute carrier organic anion transporter family member 1B1 (*SLCO1B1*), which play a crucial role in drug metabolism and hepatotoxicity risk during TB treatment (https://www.pharmgkb.org/). Among these, six genes (ATP/GTP binding protein-like 4 [*AGBL4*], *NAT2, GSTP1, SLCO1B1, Nitric oxide synthase* [*NOS*], and Cytochrome P450 family 2 subfamily B member 6 [*CYP2B6*]) demonstrated significant frequency differences across the studied populations, warranting further investigation (Tables 1 and 2), additional SNPs without significant differences are reported in Tables S2 and S3, providing a broader view of genetic variability related to TB drug metabolism in native Peruvian populations.

For *AGBL4*, SNPs *rs393994, rs319952*, and *rs320003* showed significant variations. The heterozygous genotype (AG) was the most frequent across all subpopulations. However, the AA genotype, associated with hepatotoxicity risk, was significantly more frequent in the Andean and Coastal populations compared to the Amazonian population. Despite this, the Amazonian population exhibited a higher prevalence of hepatotoxicity risk overall (OR = 3.83, $p = 0.004$).

NAT2 genotyping identified the presence of *rs4646244, rs1799929, rs1799930, rs1799931*, and *rs1495741* SNPs. Statistically significant differences were observed for *rs1799929* and *rs1799931*. The homozygous genotype (AA) for *rs1799931* was more frequent in the Andean (50.5%) and Coastal (51.2%) populations compared to the Amazonian population (40.6%). Meanwhile, the TT genotype for rs1799929 was most prevalent in the Amazonian population (78.3%), followed by the Andean (67.7%) and Coastal (55.8%) populations.

For *GSTP1*, the *rs1695* SNP showed significant variation. The homozygous AA genotype was significantly less frequent in the Amazonian population (36.2%) compared to the Andean (57.6%) and Coastal (59.3%) populations. The heterozygous AG genotype was more common in the Amazonian group (44.9%). Hepatotoxic genotypes were significantly more frequent in the Amazon population compared to the Coast (OR = 3.76, $p = 0.012$).

TABLE 1 | Genotypic frequencies of anti-TB drug metabolism genes with statistically significant differences among native Peruvian populations.

		N (%) in populations			
	Overall	**Amazon**	**Andes**	**Coast**	
Gene/SNP	***N* = 254**	***N* = 69**	***N* = 99**	***N* = 86**	***p***
AGBL4					
rs393994					
GG	56 (22.0)	22 (31.9)	18 (18.2)	16 (18.6)	**0.026**
GA	146 (57.5)	41 (59.4)	58 (58.6)	47 (54.7)	
AA	52 (20.5)	6 (8.7)	23 (23.2)	23 (26.7)	
rs319952					
GG	57 (22.4)	22 (31.9)	18 (18.2)	17 (19.8)	**0.029**
GA	145 (57.1)	41 (59.4)	58 (58.6)	46 (53.5)	
AA	52 (20.5)	6 (8.7)	23 (23.2)	23 (26.7)	
rs320003					
AA	60 (23.6)	22 (31.9)	21 (21.2)	17 (19.8)	**0.045**
AG	142 (55.9)	41 (59.4)	55 (55.6)	46 (53.5)	
GG	52 (20.5)	6 (8.7)	23 (23.2)	23 (26.7)	
NAT2					
rs1799929					
TT	169 (66.5)	54 (78.3)	67 (67.7)	48 (55.8)	**0.008**
TC	73 (28.7)	14 (20.3)	24 (24.2)	35 (40.7)	
CC	12 (4.7)	1 (1.4)	8 (8.1)	3 (3.5)	
rs1799931					
AA	122 (48.0)	28 (40.6)	50 (50.5)	44 (51.2)	**0.028**
AG	101 (39.8)	26 (37.7)	37 (37.4)	38 (44.2)	
GG	31 (12.2)	15 (21.7)	12 (12.1)	4 (4.7)	
GSTP1					
rs1695					
AA	133 (52.4)	25 (36.2)	57 (57.6)	51 (59.3)	**0.010**
AG	95 (37.4)	31 (44.9)	34 (34.3)	30 (34.9)	
GG	26 (10.2)	13 (18.8)	8 (8.1)	5 (5.8)	
SLCO1B1					
rs4149032					
TT	121 (47.6)	53 (76.8)	28 (28.3)	40 (46.5)	**< 0.001**
TC	98 (38.6)	11 (15.9)	54 (54.5)	33 (38.4)	
CC	35 (13.8)	5 (7.2)	17 (17.2)	13 (15.1)	
NOS					
rs11080344					
TT	132 (52.0)	39 (56.5)	42 (42.4)	51 (59.3)	**0.018**

(Continues)

TABLE 1 | (Continued)

Gene/SNP	Overall *N* = 254	*N* (%) in populations: Amazon *N* = 69	Andes *N* = 99	Coast *N* = 86	*p*
TC	103 (40.6)	29 (42.0)	44 (44.4)	30 (34.9)	
CC	19 (7.5)	1 (1.4)	13 (13.1)	5 (5.8)	
CYP 2B6					
rs3745274					
TT	67 (26.4)	29 (42.0)	19 (19.2)	19 (22.1)	**0.012**
TG	132 (52.0)	30 (43.5)	57 (57.6)	45 (52.3)	
GG	55 (21.7)	10 (14.5)	23 (23.2)	22 (25.6)	

Note: Bold indicates the statistical significance of $p < 0.05$.

Analysis of *SLCO1B1* revealed significant differences for *rs4149032*. The homozygous TT genotype was most frequent in the Amazonian population (76.8%), followed by the Coastal (46.5%) and Andean (28.3%) populations. The prevalence of the hepatotoxic CC genotype was significantly higher in the Amazonian group (OR = 3.81, $p = 0.012$) and lower in the Andean group (OR = 0.45, $p = 0.010$).

For *NOS*, *rs11080344* was the key SNP, showing significant differences. The homozygous TT genotype was most frequent in the Coastal population (59.3%), followed by the Amazonian (56.5%) and Andean (42.4%) populations. The heterozygous TC genotype, associated with reduced hepatotoxicity risk, was more frequent in the Andean population (44.4%), which had a significantly lower risk of hepatotoxicity compared to the Coast (OR = 0.51, $p = 0.022$).

Finally, *CYP2B6* genotyping identified *rs3745274* as the primary SNP of interest. The heterozygous TG genotype was the most frequent across all populations. However, the TT genotype, which has been linked to increased hepatotoxicity risk, was significantly higher in the Amazonian population (42.0%) compared to the Andean (19.2%) and Coastal (22.1%) populations ($p = 0.012$).

The Amazonian population exhibited a higher risk of developing DILI due to hepatotoxic alleles in the *AGBL4, GSTP1, SLCO1B1*, and *NOS* genes. In contrast, Andean populations showed a genetic profile associated with lower hepatotoxicity risk. RFMIX v2 analysis identified distinct ancestry proportions among the studied groups. The Andean population exhibited higher Native American ancestry, while the Coastal group had greater European admixture. These ancestry differences were reflected in pharmacogenetic variations, particularly in *NAT2* and *GSTP1*. These findings highlight the genetic variability underlying TB drug metabolism and the importance of tailoring treatments to specific subpopulations.

4 | Discussion

In this study, we identified differences in the prevalence of several genetic variants that affect TB drug metabolism across geographically distinct native non-admixed Peruvian populations. The Amazonian population demonstrated a higher prevalence of hepatotoxic alleles in the *AGBL4, GSTP1*, and *SLCO1B1* genes. Variants such as rs393994 in *AGBL4* were associated with an increased frequency of high-risk genotypes (OR = 3.83, $p = 0.004$). Similar findings have been reported in other studies, linking these variants to impaired cellular deglutamylation processes, which may exacerbate liver toxicity when exposed to drugs like rifampin [24]. In contrast, the Andean population exhibited a lower prevalence of DILI-associated alleles in the *NOS* gene, particularly rs11080344. This variant has been linked to reduced nitric oxide production, which may mitigate oxidative stress and hepatotoxicity [25].

Several previous studies have explored the role of genetic polymorphisms in drug metabolism, particularly in relation to *NAT2* and its impact on isoniazid metabolism. The *NAT2* gene has been widely studied in different populations, with significant variability observed among ethnic groups. Studies in East Asian populations, for instance, have shown a higher prevalence of fast acetylator alleles, whereas South American Indigenous populations tend to exhibit a higher frequency of slow acetylators, increasing their risk of DILI [8, 11]. Similarly, genetic studies in African and European populations have reported diverse acetylation patterns that influence TB treatment outcomes [15, 16].

While previous studies have identified associations between pharmacogenetic variants and anti-TB drug response in global and mestizo populations, our study provides novel insights specific to non-admixed native Peruvian subpopulations. This is the first study to examine and compare the distribution of high-risk genotypes across the Amazonian, Andean, and Coastal native groups using genomic data from individuals with > 95% Native American ancestry. We report previously undocumented population-specific differences in key pharmacogenes such as *NAT2, GSTP1, SLCO1B1, NOS, AGBL4*, and *CYP2B6*.

The *NAT2* gene plays a critical role in the metabolism of isoniazid. Variants such as *rs1799931* associated with slow acetylation phenotypes were more prevalent in the Amazonian population. Slow acetylators are at an increased risk of DILI due to

TABLE 2 | Association between native subpopulations and high-risk genotypes with statistically significant differences.

	Hepatotoxicity high risk genotype				Total				
	N	**%**	***N***	**%**	***N***	**%**	**OR**	**CI (95%)**	***p***
AGBL4—Rifampicin									
rs393994	Yes (AA/AG)		No (GG)						
Coast	63	73.3	23	26.7	86	33.9	Ref		
Andean	76	76.8	23	23.2	99	39.0	1.21	0.59–2.48	0.582
Amazon	63	91.3	6	8.7	69	27.1	3.83	1.38–12.20	**0.004**
rs319952	Yes (AA/AG)		No (GG)						
Coast	63	73.3	23	26.7	86	33.9	Ref		
Andean	76	76.8	23	23.2	99	39.0	1.21	0.59–2.48	0.582
Amazon	63	91.3	6	8.7	69	27.1	3.83	1.38–12.20	**0.004**
rs320003	Yes (GG/GA)		No (AA)						
Coast	63	73.3	23	26.7	86	33.9	Ref		
Andean	76	76.8	23	23.2	99	39.0	1.21	0.59–2.48	0.582
Amazon	63	91.3	6	8.7	69	27.1	3.83	1.38–12.20	**0.004**
High-Risk from 1000 Genomes Project Frequencies (%): PEL: 80.0, EUR: 75.0, EAS: 70.0, AFR: 65.0									
GSTP1—Pyrazinamide									
rs1695	Yes (AA)		No (AG/GG)						
Coast	5	5.8	81	94.2	86	33.9	Ref		
Andean	8	8.1	91	91.9	99	39.0	1.42	0.39–5.75	0.547
Amazon	13	18.8	56	81.2	69	27.1	3.76	1.16–14.13	**0.012**
High-Risk from 1000 Genomes Project Frequencies (%):PEL: 10.0, EUR: 5.0, EAS: 5.0, AFR: 10.0									
SLCO1B1—Rifampicin									
rs4149032	Yes (CC)		No (TT/TC)						
Coast	40	46.5	46	53.5	86	33.9	Ref		
Andean	28	28.3	71	71.7	99	39.0	0.45	0.24–0.87	**0.010**
Amazon	53	76.8	16	23.2	69	27.1	3.81	1.80–8.23	**0.012**
This SNP has a minor allele frequency (MAF) according to the 1000 Genomes database									
NOS —Ethambutol									
rs11080344	Yes (CC)		No (TT/TC)						
Coast	51	59.3	35	40.7	86	33.9	Ref		
Andean	42	42.4	57	57.6	99	39.0	0.51	0.27–0.95	**0.022**
Amazon	39	56.5	30	43.5	69	27.1	3.89	0.45–1.78	0.727
This SNP is not available in the 1000 Genomes Project									

Note: Bold indicates the statistical significance of $p < 0.05$.

the accumulation of toxic metabolites [26]. The findings from our study align with previous research indicating a higher frequency of slow acetylator alleles, such as rs1799931-AA, among South American Indigenous populations [27, 28]. The presence of *NAT25, NAT26*, and *NAT27* alleles has been consistently linked to slow metabolism in multiple populations, including Peruvians [29]. Studies in Latin American mestizo populations have demonstrated distinct metabolic profiles compared to Indigenous groups, reinforcing the importance of population-specific pharmacogenomic studies [30]. Moreover, in Peruvian TB patients, *NAT25B* and *NAT27B* were associated with higher DILI risk in mestizos, whereas *NAT25G* and *NAT213A* were protective in native populations, suggesting an evolutionary role in drug metabolism adaptations [31].

The *GSTP1* gene, responsible for detoxification and oxidative stress regulation, also showed population-specific differences. The *rs1695* AA genotype, more common in the Amazonian population, has been linked to reduced glutathione S-transferase activity and an increased risk of oxidative damage, corroborating findings from prior research [32–35]. Similarly, polymorphisms in the *SLCO1B1* gene, particularly *rs4149032*, were significantly associated with DILI in the Amazonian population (OR = 3.81, $p = 0.012$). This gene plays a vital role in hepatic transport of rifampicin, and its variants, such as rs4149032, have been associated with altered plasma concentrations of rifampicin and an increased risk of hepatotoxicity [36–38].

Interestingly, no significant differences were observed in the *CYP2B6* gene across the three subpopulations. While *CYP2B6* has been implicated in the metabolism of drugs such as efavirenz, its limited variability in this study suggests that other genetic factors may play a more critical role in influencing DILI risk among these populations [39–41]. Previous research has highlighted the impact of *CYP2E1* polymorphisms on drug metabolism, particularly in populations with a high burden of TB. Tang et al. [10] found that individuals carrying *CYP2E11D* alleles had significantly altered enzyme activity, affecting isoniazid metabolism and hepatotoxicity risk. Similar findings have been reported in South American and Asian populations, suggesting a potential genetic basis for inter-individual variability in drug response.

These findings reinforce the critical role of genetic diversity in influencing treatment outcomes. The genetic differentiation observed between the Andean and Amazonian populations is consistent with prior studies showing that geographic and cultural isolation has shaped distinct genetic profiles in South America [42–45]. Genetic ancestry plays a central role in population pharmacogenomics [46]. In a previous study, we identified the presence of adverse reactions during anti-tuberculosis treatment in the Peruvian population. We reported that 30% of the Peruvian population is associated with the slow metabolism of isoniazid [29]. We also identified haplotypes with divergent associations with DILI, based on the mestizo or native Peruvian population. For instance, we found evidence of *NAT2*5B* and *NAT2*7B* being associated with DILI risk in mestizos, while no such association has been observed in natives. Additionally, haplotypes *NAT2*5G* and *NAT2*13A* have only been negatively associated with DILI in the studied Native Peruvians [30]. Another study revealed that this environmental and genetic differentiation between the Andean and Amazonian populations has allowed natural selection and other evolutionary forces to act over millennia, shaping differences in the frequencies of genetic variants, including genes related to the immune response (*CD45* and *DUOX2*), with thyroid (*DUOX2*), cardiovascular (*HAND2-AS1*) and hematological (*TMPRSS6*) functions 4, as well as genes related to drug response [31].

Our findings suggest that there are differences in the *AGBL4, NAT2, GSTP1, SLCO1B1, NOS*, and *CYP2B6* genes between the native populations in Peru that is correlated with clinical reports about toxicity and treatment failure in Peruvian populations [47–49]. These differences could have implications for the risk of hepatotoxicity associated with the use of antituberculosis drugs [50, 51]. This study has several strengths, including its focus on underrepresented native Peruvian populations, providing crucial insights into genetic variability affecting TB drug metabolism. The comprehensive analysis of 23 genes using advanced genotyping techniques ensures high data quality while including diverse subpopulations (Coast, Andes, Amazon) and offers valuable comparative perspectives. Clinically relevant findings, such as the prevalence of hepatotoxic alleles, have practical implications for personalized TB treatment strategies.

However, the study also has limitations, such as a relatively small sample size that may restrict generalizability, the exclusion of admixed populations, and reliance on a cross-sectional design, which limits the ability to assess long-term impacts. Understanding these genetic variations is crucial for designing effective TB treatment regimens. The differences observed in this study align with previous findings that Indigenous South American populations exhibit distinct pharmacogenetic profiles compared to mestizo, European, African, and Asian populations. These findings further support the need for incorporating pharmacogenomics into TB treatment guidelines to reduce adverse effects and improve therapeutic outcomes [18, 34].

While our study identifies significant differences in allele frequencies among native Peruvian subpopulations, clinical studies are necessary to validate their impact on drug metabolism and treatment outcomes. Functional validation through pharmacokinetic analyses will determine if these variations influence tuberculosis treatment efficacy and adverse drug reactions. Only through clinical confirmation can these findings contribute to pharmacogenetic guideline development. Our results provide a foundation for future research but require further investigation in a clinical setting.

5 | Conclusions

Although our study results provide valuable insights into the frequency of metabolizing genotypes for anti-TB drugs in Peru, particularly among native populations, a deeper understanding of the factors associated with these genotypes is needed. Longitudinal studies including large samples have revealed that genetic polymorphisms play an important role in drug metabolism. Despite the limitations of being a secondary study, our findings suggest that native Peruvian subpopulations exhibit distinct metabolizing profiles associated with variants in the *AGBL4, NAT2, GSTP1, SLCO1B1, NOS*, and *CYP2B6* genes.

Author Contributions

Study design: L.J.-V. Performed the experiments: L.J.-V., M.K.H. Analyzed the data: L.J.-V., M.K.H., C.M.L., H.G. All authors have read and approved the final manuscript.

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Ethics Statement

Our study was approved by the Ethics in Research Committee of the Peruvian National Institute of Health and follows the principles of the Declaration of Helsinki.

Consent

Written informed consent was obtained from all the participants.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding authors H.G and L.J.-V., upon reasonable request.

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