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Association of *TNFα* and *IL10* polymorphisms with pulmonary tuberculosis susceptibility in an admixed population from a high-burden region in Northeast Brazil

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ABSTRACT. Pulmonary tuberculosis (PTB) remains a major cause of morbidity and mortality in developing and high-burden countries, such as Brazil. The course of *Mycobacterium tuberculosis* infection is regulated by mainly two antagonistic cytokines that are produced and secreted by T cells, TNF-α and IL-10. Differences in *TNFα* and *IL10* genes expression and protein production affected by single nucleotide polymorphisms (SNPs) have been demonstrated influence PTB susceptibility. Therefore, this study aimed to evaluate possible association between the functional polymorphisms of *TNFα* (-308G/A, rs1800629) and *IL10* (-1082A/G, rs1800896) cytokines with susceptibility to active PTB. A case-control study was carried out of 71 patients with active pulmonary tuberculosis (PTB+ group), 105 patients with nonspecific lung disease (TB-1 and TB-2 groups) and 101 clinically healthy individuals (healthy control) from reference public health hospitals in Pernambuco state (Northeast Brazil), to explore the association of cytokine polymorphisms,

sociodemographic, clinical, and epidemiological variables with developing of PTB. *TNFα* -308G/A and *IL10* -1082A/G genotyping was performed by TaqMan qPCR system. The univariate and logistic regression analyses demonstrated statistically association of male sex (OR=13.741, *P*<0,001) and age (OR=1.030, *P*=0.017) with PTB susceptibility, as risk factors. Regarding genetic variables, *IL10* -1082 G variant allele, AG and GG genotypes were also associated with developing of PTB as risk factors and maintained associated in the logistic regression dominant model (OR=7.998, *P*<0.001). Significant association was also observed between *TNFα* -308 GA genotype and PTB susceptibility, but as protective factor in univariate analysis (OR=0.375, *P*=0.005), and logistic regression dominant model associated the genotypes carrying A variant allele (OR=0.386, *P*=0.021). The study showed important role of *IL10* and *TNFα* SNPs in developing PTB in a Brazilian population, being possible biomarkers for tuberculosis susceptibility.

Key words: *Mycobacterium tuberculosis*; SNP; Interleukin 10; Tumor Necrosis Factor alpha; PTB

INTRODUCTION

Tuberculosis (TB) remains a major cause of morbidity and mortality in developing countries, such as Brazil, one of the high-burden countries for TB and established by World Health Organization (WHO) as a priority country for reducing TB incidence. In Brazil, Pernambuco state reported the third-highest incidence of TB in 2022, with five thousand new cases, and the second-highest mortality rate for the disease (3.8 deaths per 100 thousand inhabitants) (Brasil, 2023). TB incidence has also increased in developed countries. A quarter of the global population is infected with *Mycobacterium tuberculosis* (Mtb), but only about 5% of individuals develop active disease in the first year of infection (primary tuberculosis) and other 5% at some stage of their life (reactivation tuberculosis) (WHO, 2022). In these individuals, the disease progression will depend on the ability of their immune system in controlling bacilli multiplication. The main clinical form of tuberculosis is characterized by pulmonary involvement, affecting 80-85% of cases (Marseglia et al., 2019; Alsayed and Gunosewoyo, 2023).

TB is a chronic granulomatous disease caused by *M. tuberculosis*. Several factors are involved in its pathogenesis, such as environmental, social, and genetic background of infected individuals. The tuberculosis transmission occurs by air, when a person inhales droplet nuclei containing *M. tuberculosis*, and the droplet nuclei traverse the mouth or nasal passages, upper respiratory tract, and bronchi to reach the alveoli of the lung (Alsayed and Gunosewoyo, 2023). Soon after the establishment of infection, the host alveolar macrophages phagocytize mycobacteria, in which proinflammatory cytokines are synthesized, such as tumor necrosis factor alpha (TNF-α), playing a key role in the immune response of resistance against bacillus (Rastogi and Briken, 2022).

Cytokines produced in the lung, after interactions between T lymphocytes and infected macrophages, are essential to the control of tuberculosis (Marseglia et al., 2019;

Rastogi and Briken, 2022). Studies have demonstrated that host genetic factors play an important role in susceptibility / resistance to pulmonary tuberculosis (PTB) (Lima et al., 2016; Chen and Ma, 2020). Therefore, the identification of host genes responsible for susceptibility and resistance to TB should provide a significant contribution to better understand the immunopathogenesis and may lead to the development of prophylactic measures and treatment strategies (Alsayed and Gunosewoyo, 2023).

The *M. tuberculosis* infection course is regulated by two distinct patterns of cytokine produced and secreted by T cells. Th1 cells produce the cytokines INF-γ, IL12, IL6, IL1, TNF-α, which are associated with resistance to infection. These cytokines activate the microbicidal mechanisms of macrophage and participate in the tuberculosis granuloma formation (Etna et al., 2014; Marseglia et al., 2019). TNF-α is involved in the granuloma organization and control, and in synergism with IFN-γ induces the nitric oxide production, a potent antimycobacterial agent. In addition, TNF-α can stimulate cell migration, adhesion molecules expression and chemokines production (Rastogi and Briken, 2022). On the other hand, the cytokines produced by Th2 (IL4, IL10 and IL13) inhibit the proinflammatory cytokines production through its action on Th1 cells and macrophages. It is important that proinflammatory responses are controlled to minimize tissue damage in tuberculosis. In this sense, interleukin 10 (IL10) is a regulatory cytokine that inhibits inflammatory responses type 1. This cytokine plays a central role during the latent / chronic stage of PTB, and its increase has been associated with the tuberculosis reactivation (Cavalcanti et al., 2012; Marseglia et al., 2019).

Differences in cytokine production during the immune response against *M. tuberculosis* may affect Th1/Th2 balance associated with the development of active disease (Joshi et al., 2015). Several studies have indicated the important role of TNF-α and IL-10 in the tuberculosis immunopathogenesis (Liu et al., 2015; Yi et al., 2015; Yu et al., 2019). These studies have shown that single nucleotide polymorphisms (SNPs) in the promoter regions of *TNFα* and *IL10* genes significantly influence cytokines production and immune response, being involved in susceptibility, severity, and clinical evolution of tuberculosis in different populations. However, there is conflicting evidence of polymorphism's association in *TNFα* (rs1800629) and *IL10* (rs1800896) genes with active PTB in patients from some admixed human population (Milano et al., 2016).

Therefore, this study aimed to evaluate a possible association between the functional polymorphisms of *TNFα* (-308G/A, rs1800629) and *IL10* (-1082A/G, rs1800896) cytokines genes, sociodemographic, clinical, and epidemiological variables with susceptibility to active pulmonary tuberculosis in patients from Pernambuco state, an admixed Brazilian population.

MATERIAL AND METHODS

Study population and groups classification

This case-control study consisted of 176 patients with respiratory symptomatology (85 females and 96 males) and 101 (83 females and 18 males) clinically healthy individuals from reference public health hospitals in Pernambuco state (Northeast Brazil). The general inclusion criteria to patients were both sex, respiratory symptoms, and TB suspicion. The general exclusion criteria were seropositivity for HIV infection since it is a risk factor for

PTB development. All patients were classified according to TB diagnosis, which is based on clinical symptoms, laboratorial and imaging exams. Therefore, 71 patients who were diagnosed and treated for PTB, negative for HIV infection (BioPix – HIV 1 & 2) were included in the case group (PTB+). The PTB diagnosis was based on sputum smear positivity, sputum culture positivity, clinical-radiological exams, and anti-TB therapeutical response (Brasil, 2022).

The control groups consisted of three profiles. A group of 105 voluntary patients, who were diagnosed and treated with nonspecific lung disease, were stratified in two subgroups: a subgroup with 53 patients (control group 1 – TB-1) positive for tuberculin skin test (≥10mm); and other subgroup with 57 patients (control group 2 – TB-2) negative for tuberculin skin test (<10mm). The third group consisted of 101 clinically healthy subjects (control group 3 – healthy control) that was negative for PTB, showed no clinical or laboratorial history of tuberculosis, normal posteroanterior (PA) chest X-ray, presence of Bacille Calmette-Guérin (BCG) vaccine scar, no respiratory symptoms, and negative for HIV infection (BioPix – HIV 1 & 2).

Sociodemographic, clinical, epidemiological and laboratory data were collected from medical records (age, sex, history of smoking and alcoholism, comorbidity (hypertension and diabetes mellitus), presence of BCG vaccine scar, history of previous treatment for tuberculosis, and diagnostic test used) to investigate the increased risk of PTB. All individuals answered standard questionnaires, gave written informed consent, and provided blood samples for genetic analysis. The study was approved by the IAM/FIOCRUZ Ethics Committee (protocol number CAAE: 0056.0.095.000-11).

Single base polymorphisms (SNPs) selection

The SNPs rs1800896 (*IL10* -1082A/G) and rs1800629 (*TNFα* -308G/A) were selected based on the literature, functional characteristics, and minor allele frequency (MAF ≥10%) in European, Amerindian, and African populations, which reflect the Brazilian genetic admixture (Kehdy et al., 2015). SNPs selection was performed using NCBI dbSNP (https://www.ncbi.nlm.nih.gov/snp/) and Ensembl (http://www.ensembl.org/index.html) databases.

Blood collection and DNA extraction

The blood samples were collected from all subjects in EDTA tubes (4mL). Genomic DNA was extracted from whole blood using QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. DNA quantification and purity were assessed using the NanoDrop 2000 (ThermoFisher) spectrophotometer, considering 260/280nm and 260/230nm absorbance ratios. DNA were stored at −20ºC until real-time PCR analyses.

SNPs genotyping

The SNPs genotyping of the *IL10* (-1082A/G, rs1800896) and *TNFα* (-308G/A, rs1800629) genes was performed by TaqMan SNP Genotyping Assay Methods (Applied Biosystems): (C_1747360_10) and (C_7514879_10), respectively. The amplification

reactions were carried out on the ABI 7500 Real-Time PCR System (Applied Biosystems) using protocols conditions recommended by the manufacturer's instructions.

Statistical analysis

The allelic and genotypic frequencies of -1082 A/G *IL10* and -308 G/A *TNFα* SNPs were assessed by direct counting, and conformity with Hardy-Weinberg equilibrium (EHW) was evaluated using the X^2 test. Sample size analyses were performed in the G*Power software, version 3.1.9.7, using post-roc power test. Fisher exact test was used to assess whether genetic, sociodemographic, epidemiological, and clinical variables were associated with susceptibility to PTB. Student's *t*-test was used to compare the groups for variable age, which follow a normal distribution (according to Shapiro-Wilk test). The variables deemed to have clinical importance and reached p-value ≤0.20 during univariate analyses were included in the logistic regression analysis. All tests were two-tailed, and the significance level (α) was set at $p<0.05$. The statistical analyses were performed using R software, version 4.2.0.

RESULTS

Analyses of sociodemographic, epidemiological, clinical, and laboratorial data

Multiple factors (sociodemographic, epidemiological, clinical, and laboratorial variables) were evaluated in this study to investigate their influence on susceptibility to PTB (Supplementary Table). No statistical differences were observed when comparing PTB+ and TB- 1/TB- 2 groups during univariate analyses for smoking (*P*=0.846) or alcoholic habits (*P*=0.219), neither diabetes mellitus (*P*=1.00) or systemic arterial hypertension (*P*=0.610) comorbidities. We observed that men were more frequent in the PTB+ group (64.8%) than TB- groups (45%), showing twice higher risk to develop pulmonary tuberculosis compared to women (OR=2.198; 95%CI=1.144–4.291; *P*=0.014). Age was slightly higher in the TB- groups (48 and 51 years old) than PTB+ group (43 years old) with statistical difference (*P*=0.007). When compared age of PTB+ group (43.1±15.2) with healthy control group (38±16.7), we also observed a statistically significant difference (*P*=0.040).

Genetic association analysis

The genotype distribution of -1082 A/G *IL10* and -308 G/A *TNFα* polymorphisms was in conformity to Hardy-Weinberg equilibrium in all studied groups. The post-hoc power tests for the two SNPs were > 80%, thus sample size has been shown representative for the study. The G allele frequency of *IL10* (-1082 A/G) was similar in PTB+ case group (46.5%) compared to TB- 1 (47.2%) and TB- 2 (41.2) controls groups, with no statistical difference (*P*=1.00 and *P*=0.448, respectively). However, it was less frequent in the healthy control group (23.8%), and when compared with PTB+ group, we observed a statistically significant difference, associating the G variant allele with pulmonary tuberculosis susceptibility as a risk factor (OR=2.777, 95%CI=1.710–4.544, *P*<0.001). The same was observed when comparing PTB+ and all controls groups (OR=1.658, 95%CI=1.105–2.484,

P=0.012). In relation to genotype containing the G variant allele (AG and GG), both were also associated with PTB susceptibility. Although the AG and GG genotype frequency was close in PTB+, TB- 1 and TB- 2 groups (Table 1), these genotypes were considerably less frequent in healthy control group, mainly for GG genotype (33.7% and 6.9%, respectively). A patient carrying AG genotype has twice higher risk to develop PTB than a patient with AA genotype (OR=2.289, 95%CI=1.095–4.849, *P*=0.024). Moreover, if a patient has GG genotype, the risk for developing pulmonary tuberculosis increase to six-time higher (OR=6.572, 95%CI=2.262–21.242, *P*<0.001, Table 1).

Table 1. Allele and genotype frequencies of the rs1800896 *IL10* (-1082A/G) and rs1800629 *TNFα* (-308G/A) polymorphisms, and comparison among patients' groups to assess genetic association with susceptibility to pulmonary tuberculosis.

SNP	PTB+	Controls (n=211)			*p*-value; OR [95% CI] *			
	n=71 (%)	TB- 1 n=53 (%)	TB- 2 n=57 (%)	Healthy Control n=101 (%)	PTB+ vs. TB- 1	PTB+ vs. TB- 2	PTB+ vs. Healthy Control	PTB+ vs. Controls
***IL10* (-1082 A/G, rs1800896)**								
Alleles								
A	76 (53.5)	56 (52.8)	67 (58.8)	154 (76.2)	Ref	Ref	Ref	Ref
G	66 (46.5)	50 (47.2)	47 (41.2)	48 (23.8)	1; 0.972 [0.569 – 1.662]	0.448; 1.237 [0.730 – 2.101]	<0.001; 2.777 [1.710 – 4.544]	0.012; 1.658 [1.105 – 2.484]
Genotypes								
AA	23 (32.4)	18 (34.0)	22 (38.6)	60 (59.4)	Ref	Ref	Ref	Ref
AG	30 (42.3)	20 (37.7)	23 (40.4)	34 (33.7)	0.831; 1.172 [0.467 – 2.942]	0.685; 1.245 [0.521 – 2.988]	0.024; 2.289 [1.095 – 4.849]	0.116; 1.689 [0.872 – 3.311]
GG	18 (25.3)	15 (28.3)	12 (21.0)	7 (6.9)	1; 0.939 [0.339 – 2.612]	0.486; 1.428 [0.511 – 4.081]	<0.001; 6.572 [2.262 – 21.242]	0.031; 2.289 [1.032 – 5.057]
***TNF* (-308 G/A, rs1800629)**								
Alleles								
G	107 (75.4)	78 (73.6)	82 (71.9)	139 (68.8)	Ref	Ref	Ref	Ref
A	35 (24.6)	28 (26.4)	32 (28.1)	63 (31.2)	0.769; 0.912 [0.493 – 1.695]	0.569; 0.839 [0.462 – 1.527]	0.225; 0.722 [0.430 – 1.201]	0.332; 0.795 [0.498 – 1.249]
Genotypes								
GG	44 (62.0)	31 (58.5)	32 (56.1)	44 (43.6)	Ref	Ref	Ref	Ref
GA	19 (26.8)	16 (30.2)	18 (31.6)	51 (50.5)	0.684; 0.838 [0.346 – 2.041]	0.549; 0.769 [0.324 – 1.826]	0.005; 0.375 [0.179 – 0.766]	0.055; 0.545 [0.279 – 1.035]
AA	8 (11.2)	6 (11.3)	7 (12.3)	6 (5.9)	1; 0.940 [0.256 – 3.640]	0.781; 0.833 [0.236 – 3.001]	0.775; 1.329 [0.369 – 5.063]	1; 1.024 [0.359 – 2.676]

*Genetic association by Fisher exact test. CI: confidence interval; OR: Odds Ratio; PTB: pulmonary tuberculosis; TB: tuberculosis.

Regarding *TNFα* polymorphism (-308 G/A), the A variant allele frequency was similar in three groups (PTB+=24.6%; TB-1=26.4%; and TB-2=28.1%), showing no association with pulmonary tuberculosis (PTB+ vs TB-1: *P*=0.769; PTB+ vs TB-2: *P*=0.569), and slightly higher in the healthy control group, but with no statistical difference compared to PTB+ group (*P*=0.225). When comparing PTB+ and all controls groups, we also observed no statistical association (*P*=0.332). In relation to GA and AA genotype, we found a significant association with PTB only for heterozygous GA, when comparing PTB+ with healthy control group. Since the GA genotype was significantly more frequent in the healthy group (50.5%) than PTB+ (26.8%), almost twice, it was associated as a protective factor to pulmonary tuberculosis susceptibility (OR=0.375, 95%CI=0.179–0.766, *P*=0.005). The TB- 1 and TB- 2 groups showed slightly higher GA (30.2% and 31.6%, respectively) genotype frequency than PTB+ group (26.8%), but with no statistical association (*P*=0.684 and *P*=0.549). These reflected in the statistical trend observed when comparing PTB+ with

all controls (OR=0.545, 95%CI=0.279–1.035, *P*=0.055). For the AA genotype, PTB+, TB-1 and TB- 2 groups showed similar frequency (Table 1).

Logistic regression analysis

Since the epidemiological and clinical variables have not achieved p-value ≤0.20 in univariate analyses (Supplementary Table), they were not included in the logistic regression analyses. Therefore, a logistic regression model was performed only with sociodemographic variables (age and sex), which were previous associated, and genetic factors (*TNFα* -308 G/A and *IL10* -1082 A/G). The results maintained significant association between age (OR=1.03, 95%CI=1.01–1.06, *P*<0.0167), and male sex with thirteen-times higher risk for developing PTB (OR=13.74, 95%CI=5.79–32.59, *P*<0.0001, Table 2). In addition, association of *IL10* -1082 A/G polymorphism with PTB was also maintained, being linked with an eight times higher risk (OR=8.00, 95%CI=2.72–23.52, *P*=0.0002). Moreover, the logistic regression model associated *TNFα* -308 G/A polymorphism as a protective factor with PTB susceptibility (OR=0.39, 95%CI=0.17–0.87, *P*=0.0208).

Table 2. Variables included in a fitted logistic regression model to explain susceptibility to pulmonary tuberculosis.

Variables	Estimate (β)*	OR	95% CI	*P*
-308 G/A (*TNFα*) genotypes [a]	-0.95125	0,3863	0,1724 – 0.8654	**0,0208**
-1082 A/G (*IL10*) genotypes [b]	2.07913	7,9975	2,7195 – 23.5192	**0,0002**
Male sex	2.2035	13,7405	5,7935 – 32.5887	**<0,0001**
Age	0.02927	1,0297	1,0053 – 1.0547	**0,0167**
(intercept)	-0.39972	_	_	0,5755

CI: confidence interval; OR: odds ratio. [a] GG versus GA and AA genotypes. [b] AA versus AG and GG genotypes. *Model's internal validation: AUROC=0.8513; Z=0.1204; *P*=0.9042.

Considering a hypothetical example, but typical in our population sample with these associated factors, a 38 years-old female patient with *IL10* -1082 AA (ancestral) and *TNFα* -308 AA (protective variant) genotypes would have 44% of chance for developing PTB. However, if it was a 43 years-old male patient with *IL10* -1082 GG (risk variant) and *TNFα* -308 GG (ancestral) genotypes the chance increase to almost 99%. The model's internal validation showed that the analysis had good quality and adherence (AUROC=0.8513; Z=0.1204; *P*=0.9042) thus being appropriated to do predictions for pulmonary tuberculosis susceptibility.

DISCUSSION

The developing of PTB is based on several risk factors, mainly that related to socioeconomic, behavioral, and biologic aspects (Brasil, 2023; WHO, 2022). Thus, this study performed multiple factors analyses, including sociodemographic, clinical, epidemiological, and genetic variables, in an admixed Brazilian population to evaluate their possible association with PTB susceptibility. Regarding that, our study showed statistical difference of male sex and age among the groups studied, indicating that risk factors affect

more adult men than women during *M. tuberculosis* infection. It has been related to intrinsic sex aspects, such as habits and lifestyle, which promote Mtb bacillus exposition, thus, increasing disease incidence in men individuals (CDC, 2021). These results corroborate with the literature, which demonstrate that in the developing countries, such as Brazil, 80% of individuals with active tuberculosis are in age group 15-59 years old, the age group of highest social productivity, consequently, elevated exposition to Mtb (Divo et al., 2022).

The TNF-α plays an important role in immune response against tuberculosis, acting synergically with INF-γ in macrophages activation and granuloma formation during *M. tuberculosis* infection. Granuloma organization failure results in higher *M. tuberculosis* dissemination, which can cause host death whether not controlled (Cavalcanti et al., 2012; Etna et al., 2014). Some polymorphisms into promoter region of *TNFα*, have significant effect in gene transcription level, resulting in higher TNF-α gene expression and protein production (Yi et al., 2015). However, cytokine production is regulated by complex molecular pathways since increased TNF-α levels can generate tissue and organ damage. Researchers have shown that *TNFα* expression active IL-10 protein liberation, which inhibit TNF-α production, and this cytokine interaction may influence on tuberculosis susceptibility (Etna et al., 2014; Joshi et al., 2015). Therefore, several studies have been performed in different ethnic populations to evaluate the functional SNPs in *TNFα* (-308G/A) and *IL10* (-1082A/G) genes promoter region and its association with PTB (Zhu et al., 2012; Liu et al., 2015; Yi et al., 2015; Yu et al. 2019; Chen and Ma, 2020).

The *TNFα* rs1800629 polymorphism is a guanine-to-adenine transition (G > A) at promoter position -308 that has been demonstrated to increase *TNFα* expression, resulting in high protein levels (Joshi et al., 2015). Thus, corroborating with some studies (Yi et al., 2015), our results showed association of *TNFα* -308 genotype carrying A variant allele with PTB susceptibility, as a protective factor, in both analyses (univariate and multivariate logistic regression). The variant allele effect depends on genotype status, compound of homozygosity or heterozygosity. Although there are controversial reports in literature concerning *TNFα* -308G/A SNP association with PTB (Zhu et al., 2012), it is believed to affect tuberculosis susceptibility and severity. Joshi et al., evaluating serum cytokine levels of TNF-α and -308G/A polymorphism in PTB and TB- groups, showed high TNF-α levels in the patients carrying A allele and significant association of this SNP with tuberculosis susceptibility (Joshi et al., 2015). Since TNF-α is a proinflammatory cytokine that stimulate granuloma formation to control *M. tuberculosis* dissemination, its production dysregulation influences the development of PTB (Etna et al., 2014; Rastogi and Briken, 2022).

Regarding *IL10* -1082A/G polymorphism, we found significant association of G variant allele and genotypes (AG and GG) with PTB susceptibility as risk factors in univariate analyses and maintained in logistic regression model, corroborating with most studies (Liu et al., 2015; Yu et al., 2019; Chen and Ma, 2020). The *IL10* rs1800896 SNP is a nucleotide substitution of the adenine for guanine (A>G) at promoter position -1082, a putative Ets (E26 transformation-specific) transcription factor-binding site, that may affect the binding affinity of this transcriptional factor with promoter region (Areeshi et al., 2017; Yu et al., 2019). As a result, *IL10* -1082A/G polymorphism has been described to increase gene transcription, altering cytokine levels, thus, affecting Th1/Th2 balance with important implications in the development of tuberculosis (Joshi et al., 2015; Areeshi et al., 2017). Joshi and collaborators observed elevated IL-10 serum levels in individuals carrying G allele, mainly for GG genotype. The authors found *IL10* -1082A/G SNP to be involved in

the clinical tuberculosis outcome, associating genotypes carrying G variant allele with higher risk to develop pulmonary tuberculosis (Joshi et al., 2015). IL-10 is an anti-inflammatory interleukin that downregulates the expression of many cytokines, including TNF-α (Etna et al., 2014). Therefore, high IL-10 levels could significantly reduce TNF-α production, influencing the development of PTB, such as inhibiting granuloma formation, consequently, allowing *M. tuberculosis* dissemination.

Any differences regarding the polymorphism frequency can be explained by ethnic differences among the study populations (Kehdy et al., 2015). The large genetic diversity and allele distribution are influenced and generated by environment selective forces in which species evolve. In this scenario, it has been postulated the theory of heterozygosity advantage in humans, which propose heterozygous individuals are able to response in more effective way to the environmental selective pressure since they carry higher genetic variety. Therefore, heterozygous individuals may have better adaptation to adverse conditions, such as microorganism infections (Cooke and Hill, 2001; Kehdy et al., 2015). This theory could explain the higher frequency, more than 50%, of heterozygotes for *TNFα* -308G/A SNP in the healthy control group and its association with PTB susceptibility as a protective factor.

In conclusion, looking for genetic, sociodemographic, clinical, and epidemiological factors that would be associated with pulmonary tuberculosis, the study showed important role of *IL10* (-1082A/G, rs1800896) and *TNFα* (-308G/A, rs1800629) polymorphisms with age and male sex sociodemographic variables in developing pulmonary tuberculosis in an admixed population from Northeast Brazil. Even though the study was conducted in a genetically heterogeneous population, could affect association analyses, the results highlighted the significance of these SNPs, suggesting their potential as biomarkers for tuberculosis susceptibility. It also emphasized the importance of association studies in admixed populations with high disease burden. Further research is still required to better understand how these functional genetic alterations influence the course of *Mycobacterium tuberculosis* infection.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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