Research Square

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Tumor Necrosis Factor and Interleukin 10 Polymorphism in Adult Pulmonary Tuberculosis Patients from Punjab, Pakistan: A Case-Control Study

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Abstract

Background

Single nucleotide polymorphisms (SNPs) in tumor necrosis factor (*TNF*) and interleukin 10 (*IL10*) genes influence tuberculosis (TB) susceptibility and progression, which may vary depending on the population studied.

Objectives

To investigate specific genetic variants in the *TNF* and *IL10* genes among TB patients of Punjab, Pakistan.

Methods

Out of 941 TB suspect samples tested on GeneXpert MTB/RIF assay, 170 confirmed pulmonary TB cases and 156 healthy controls were selected. Genomic DNA, extracted from whole blood, was used to analyze five target SNPs including two in *TNF* (-238 G/A, -308 G/A) and three in *IL10* (-592 C/A, -819 C/T, -1082 G/A) genes via tetra-primer amplification refractory mutation system – polymerase chain reaction (ARMS-PCR). Results were confirmed using Sanger sequencing and analyzed via BioEdit and BLASTn alignment.

Results

Demographic characteristics were similar, except 19 (11.2%) patients had family history of TB ($p = < 0.001$). *TNF − 308* minor genotype distribution differed significantly between groups ($p = 0.012$). *TNF − 308* A allele showed higher chance of occurrence in TB cases (OR = 1.612; $p = 0.003$). *TNF* − 238 and *IL10* -592, -819, and − 1082 polymorphisms showed no significant difference in allele or genotype distribution ($p = > 0.05$). *TNF* AA (OR = 0.286; $p = < 0.001$) and GG (OR = 0.272; $p = < 0.001$) haplotypes showed protective effect; whereas *TNF* AG (OR = 6.988; $p = < 0.001$) and GA (OR = 157.181; $p = < 0.001$) demonstrated significant TB risk. *IL10* CCA (OR = 0.648; $p = 0.043$) haplotype also showed protective effect.

Conclusions

Genetic polymorphisms in the *TNF* and *IL10* genes are significant modulators of pulmonary TB susceptibility in people of Punjab, Pakistan.

Introduction

Tuberculosis (TB) remains a significant global public health challenge. The World Health Organization reported that approximately 10.7 million individuals contracted TB worldwide and 1.23 million died due to TB in 2024 [1]. Pakistan has an incidence of 669,000 new TB cases, with about 14,000 of these cases being drug-resistant, annually. This positions Pakistan as the fifth highest-burden country worldwide, contributing 61% to the TB burden in the WHO Eastern Mediterranean Region. Notably, the province of Punjab, the most populous in Pakistan, accounts for 60% of the nation's TB cases [2–3].

Innate immune cells initially respond to *M. tuberculosis*, influencing the infection outcome. Tumor Necrosis Factor-alpha (TNF-alpha), a pro-inflammatory cytokine, helps contain *M. tuberculosis*. Certain TNF single nucleotide polymorphisms (SNPs) can increase TNF-alpha production, enhancing the immune response but also causing excessive inflammation and tissue damage. Other TNF SNPs reduce TNF-alpha production, potentially impairing M. tuberculosis control. Interleukin-10 (IL10) is an anti-inflammatory cytokine, and SNPs increasing IL10 production can suppress the immune response, raising TB susceptibility [4]. Hence, maintaining a balance between pro-inflammatory TNF-alpha and anti-inflammatory IL10 is critical for effective infection defense [5].

SNPs in the TNF and IL10 genes have been extensively studied in relation to *M. tuberculosis* infection, as these cytokines play a crucial role in the host immune response against TB, with certain SNPs potentially influencing susceptibility to the disease and its progression [4]. However, the relationship between these SNPs and TB is complex and likely influenced by interactions with other genetic factors and environmental exposures [4]. The association between these SNPs and TB susceptibility can vary depending on the population studied [6]. Therefore, the study aimed to investigate the specific genetic variants in the TNF and IL10 genes among TB patients of Punjab, Pakistan. By analyzing these genetic variations, the study seeks to elucidate their potential impact on TB susceptibility, resistance, and disease progression, providing valuable insights for the development of targeted interventions and therapeutic strategies.

Materials and methods

The case-control study was conducted at the University of Veterinary and Animal Sciences, Lahore, Pakistan. A total of 941 TB suspect samples have been tested. Patients diagnosed with pulmonary tuberculosis, aged 18 to 60, of both sexes, and belonging to any area of Punjab, were recruited. The GeneXpert MTB/RIF assay was used to confirm pulmonary TB patients as MTB positive and healthy controls as MTB negative.

The sample size was determined using the OpenEpi calculator, considering a 2.7 risk/prevalence ratio, 95% confidence level, and 80% test power. The study group included 170 patients, while the control group included 156 age- and sex-matched healthy volunteers from the general population.

At the time of recruitment in the study, demographic data including current age (years), sex (male or female), residential area (urban or rural), and family history of TB (no or yes) were collected by using an

ainterviewer administered questionnaire. Whole blood specimens were collected for genomic DNA extraction using the spin column method [7].

Target DNA (for SNPs – 238 G/A and – 308 G/A in *TNF* gene and – 592 C/A, -819 C/T and – 1082 G/A in *IL10* gene) was amplified using the tetra-primer amplification refractory mutation system–polymerase chain reaction (ARMS-PCR). Tetra-primers were designed using the Primer1 online tool [8]. Detail about primers and PCR conditions are given in Supplementary Tables 1 & 2. DNA sequence alignment was performed using BioEdit v7.0.5 and BLASTn software [9, 10]. Upon aligning the DNA sequencing data with the reference DNA sequence of the human *TNF* regulatory region, we identified and confirmed the presence of SNPs using the associated chromatograms. The aligned sequence and chromatogram identified the polymorphisms for *TNF* at two positions i.e., *TNF – 238* (G/A) and *TNF – 308* (G/A).

SHEsis was utilized for conducting haplotype analyses, genetic linkage, and association studies at polymorphic sites (http://analysis.bio-x.cn/myAnalysis.php) [11]. The tests were conducted to compute and examine the genotypes and allelic frequencies for all chosen loci in both patients affected by TB and unaffected controls.

The frequencies of genotypes were computed using data from all sequences obtained from both TB cases and controls. The statistical analysis was conducted using the Hardy-Weinberg equilibrium (HWE) test and Fisher's exact test. The genotyping data was subjected to a test for HWE. The genotype frequencies that were observed and expected were compared using a Chi-square test. A p= exceeding 0.05 was deemed to indicate equilibrium. The equilibrium of alleles and their significance were determined by calculating their respective chi-square and p = s. Fisher's exact test was conducted to ascertain the presence of any correlation between the examined SNPs and TB. A significance level of $p < 0.05$ was used.

Results

Among 941, a total of 170 (18.1%) were GeneXpert MTB/RIF assay confirmed positive. These 170 confirmed cases and 156 healthy controls were further analyzed. Table 1 shows that demographic characteristics of TB patients, including age, sex and residential area were similar to the characteristics of controls. However, 19 (11.2%) TB patients had reported a family history of TB ($p < 0.001$).

Table 1
Demographic characteristics of TB cases versus controls

		TB Patients (N = 170)	Controls (N = 156)	p=
Age (years)	**Mean**	36.5 ± 15.2	33.6 ± 14.6	0.080
	< 40	102 (60.0%)	90 (57.7%)	0.673
	≥ 40	62 (40.0%)	66 (42.3%)	
Sex	**Male**	92 (54.1%)	81 (51.9%)	0.691
	Female	78 (45.9%)	75 (48.1%)	
Residential area	**Rural**	101 (59.4%)	85 (54.5%)	0.372
	Urban	69 (40.6%)	71 (45.5%)	
Family history of TB	**No**	151 (88.8%)	156 (100.0%)	< 0.001
	Yes	19 (11.2%)	0 (0.0%)	

ARMS-PCR products of TNF (-238 & -308) and IL10 (-592, -819, & -1082) were evaluated using agarose gel electrophoresis as shown in **supplementary Figs. 1 to 5**.

Ethanol precipitation method was employed to purify the amplicons, and DNA sequencing as shown in Figs. 1 & 2 was conducted by 1st BASE, a facility in Singapore, utilizing Sanger sequencing.

The sequencing chromatograms in Fig. 1 exhibit clear and well-defined nucleotide peaks at the targeted polymorphic loci, confirming the successful identification of SNP in the TNFA and IL10 genes. The chromatograms demonstrate the presence of both homozygous and heterozygous genotypes, reflecting allelic variation among the analyzed samples. The high signal quality and distinct base calls indicate reliable sequencing data, supporting the accuracy of SNP genotyping for subsequent genetic association analysis.

The sequencing profiles in Fig. 2, clearly demonstrate the nucleotide variations at the analyzed polymorphic sites, with distinct peaks indicating the presence of both heterozygous and homozygous genotypes. The high-quality chromatograms confirm accurate SNP detection and validate the reliability of the sequencing results used for subsequent genetic association analysis.

Genotypes frequencies of all five target SNPs are compared across TB patients and normal controls and given in Table 2. Out of five only minor genotype G/A of *TNF − 308* in TB cases was found statistically significant (p = 0.012) when compared with controls. AA genotype was notably more frequent in TB patients (25.9%) than in controls (14.1%) while GG genotype was underrepresented in cases (28.2%) as compared to controls (39.7%). The pattern suggests − 308 A allele tracks with disease status at genotype level. All other SNPs- both *TNF − 238* and three *IL10* variants, showed closely matched

genotype proportions between groups (p= ranging between 0.585–0.794), indicating no meaningful genotypic association with TB susceptibility at these loci in selected population.

Table 2
Comparison of *TNF* and *IL10* minor genotype between TB cases and controls

SNP	Genotype	N	TB patients N = 170		Controls N = 156		p=
TNF – 238 G/A	**GG**	**122**	67	39.4%	55	35.3%	0.682
	GA	**152**	78	45.9%	74	47.4%	
	AA	**52**	25	14.7%	27	17.3%	
TNF – 308 G/A	**GG**	**110**	48	28.2%	62	39.7%	0.012
	GA	**150**	78	45.9%	72	46.2%	
	AA	**66**	44	25.9%	22	14.1%	
IL10 -592 C/A	**CC**	**145**	75	44.1%	70	44.9%	0.606
	CA	**146**	74	43.5%	72	46.2%	
	AA	**35**	21	12.4%	14	9.0%	
IL10 -819 C/T	**CC**	**150**	75	44.1%	75	48.1%	0.585
	CT	**130**	68	40.0%	62	39.7%	
	TT	**46**	27	15.9%	19	12.2%	
IL10 -1082 G/A	**GG**	**126**	68	40.0%	58	37.2%	0.794
	GA	**140**	70	41.2%	70	44.9%	
	AA	**60**	32	18.8%	28	17.9%	

Allele frequencies, along owith odds ratios (OR) and 95% confidence intervals in Table 3 describe the association strength in population genetics. *TNF – 308* G/A is again a standout: the A allele was clearly more common in TB patients (48.8%) than in controls (37.2%) with an OR of 1.612 (95% CI 1.179–2.204, p = 0.003). the value of the confidence interval above 1.0 indicates a real risk association, since – 308 A allele has almost double the odds of TB as compared to G allele. The four other SNPs showed no significant association. All of their confidence intervals crossing 1.0, meaning small differences seen could just be due to chance.

Taken together, Tables 2 and Table 3, this converges on *TNF – 308* as the only locus with reproducible evidence of association at both the genotype and allele level.

Table 3
Comparison of *TNF* and *IL10* allele frequency between TB cases and controls

SNP	Allele	TB patients Allele n = 340		Controls Allele n = 312		OR (95% CI)	p=
TNF – 238 G/A	**G**	212	62.4%	184	59.0%	1	0.422
	A	128	37.6%	128	41.0%	0.867 (0.633–1.189)	
TNF – 308 G/A	**G**	174	51.2%	196	62.8%	1	0.003
	A	166	48.8%	116	37.2%	1.612 (1.179–2.204)	
IL10 -592 C/A	**C**	224	65.9%	212	67.9%	1	0.633
	A	116	34.1%	100	32.1%	1.098 (0.791–1.522)	
IL10 -819 C/T	**C**	218	64.1%	212	67.9%	1	0.342
	T	122	35.9%	100	32.1%	1.186 (0.857–1.642)	
IL10 -1082 G/A	**G**	210	61.8%	186	59.6%	1	0.630
	A	130	38.2%	126	40.4%	0.913 (0.667–1.252)	

The combined effect of SNPs into haplotypes describe more stronger effects to describe linkage disequilibrium and combinatorial risk as shown in Table 4. Two-locus haplotypes of TNF AA (OR = 0.286, 95% CI 0.195–0.418, p = < 0.001) and GG (OR = 0.272, 95% CI 0.196–.377, p = < 0.001) haplotypes showed a statistically significant protective effect for TB. Whereas, TNF AG (OR = 6.988; 95% CI 3.803–12.843, p = < 0.001) and GA (OR = 157.181, 95% CI 22.713-1087.758, p = < 0.001) demonstrated a statistically significant greater risk association of TB. On the other hand, seven of eight, three-locus haplotypes of *IL10* only CCA (OR = 0.648, CI 0.425–0.989, p = 0.043) haplotype showed a modest statistically significant protective effect for TB. Seven haplotypes (ACA, ACG, ATA, ATG, CCG, CTA, CTG) show ORs cluster near 1.0 with non-significant p = s (0.164–0.966).

Table 4
Haplotype analyses of *TNF* and *IL10* polymorphism loci

		TB patients		Controls		OR (95% CI)	p=
		Allele n = 340		Allele n = 312			
TNF	**A A**	48.56	14.3%	114.96	36.8%	0.286 (0.195–0.418)	< 0.001
	A G	79.44	23.4%	13.04	4.2%	6.988 (3.802–12.843)	< 0.001
	G A	117.44	34.5%	1.04	0.3%	157.181 (22.713-1087.758)	< 0.001
	G G	94.56	27.8%	182.96	58.6%	0.272 (0.196–0.377)	< 0.001
IL10	**A C A**	34.54	10.2%	29.96	9.6%	1.064 (0.636–1.782)	0.812
	A C G	35.20	10.4%	35.85	11.5%	0.890 (0.543–1.456)	0.641
	A T A	13.51	4.0%	12.60	4.0%	0.983 (0.449–2.153)	0.966
	A T G	32.76	9.6%	21.59	6.9%	1.434 (0.814–2.527)	0.210
	C C A	45.03	13.2%	59.46	19.1%	0.648 (0.425–0.989)	0.043
	C C G	103.23	30.4%	86.72	27.8%	1.133 (0.807–1.589)	0.471
	C T A	36.93	10.9%	23.98	7.7%	1.463 (0.854–2.508)	0.164
	C T G	38.81	11.4%	41.83	13.4%	0.832 (0.522–1.327)	0.440

Discussion

Tuberculosis remains a significant inflammatory infectious disease that poses a severe threat to global health, with host genetic factors increasingly recognized as critical determinants of susceptibility and disease progression [12, 13]. The present case-control study investigated the association between five functional SNPs in the promoter regions of TNF (-238G/A.-308G/A) and IL 10 (-592 C/A, -819 C/T, -1082 G/A) genes and pulmonary TB susceptibility in the Punjabi population of Pakistan. Our findings demonstrate that genetic variations in these cytokine genes significantly modulate TB risk, with the *TNF − 308* A allele showing a strong association with increased susceptibility. In contrast, specific haplotypes in both genes conferred either protective or risk effects.

The most striking finding of our study was the significant association between the *TNF − 308* G/A polymorphism and pulmonary TB. The distribution of the minor genotype (AA) was significantly different between cases and controls (p = 0.012), and the A allele demonstrated a 1.6-fold increased odds of TB occurrence (OR = 1.612, 95% CI 1.179–2.204, p = 0.003). This finding aligns with a growing body of evidence implicating the *TNF − 308* A allele in heightened TB susceptibility across diverse populations.

The functional significance of the − 308 polymorphisms lies in its location within the promoter region, where A allele has been associated with increased transcriptional activity and higher TNF-alpha

production compared to the G allele [14]. TNF-alpha plays a dual role in TB pathogenesis; adequate levels are essential for granuloma formation and containment of *Mycobacterium tuberculosis*, while excessive production may contribute to immunopathology and tissue damage [15]. The A allele`s association with elevated TNF-alpha production could theoretically enhance mycobacterial control; however,our findings suggest that in the Punjabi populations, this allele paradoxically increases susceptibility.This apparent contradiction may reflect the complex interplay between pro-inflammatory responses and immune-mediated pathology, where excessive inflammation creates a permissive environment for bacterial persistence.[16]

Our results are consistent with several recent investigations. A meta-analysis by Li and colleagues (2021) encompassing 42 studies with 7,894 cases and 9,232 controls found that TNF − 308 A allele was significantly associated with increased TB risk in Asian populations (OR = 1.34, 95% CI 1.12–1.61), though this association was not observed in Caucasian or African populations [17]. Similarly, Khera and colleagues (2021) reported that the TNF − 308 AA genotype conferred increased susceptibility to pulmonary TB in North Indian patients (OR = 2.14, 95% CI 1.28–3.58) [18]. More recently, Singh and colleagues (2023) demonstrated that individuals carrying the A allele exhibited significantly higher serum TNF-alpha levels and more extensive pulmonary involvement compared to GG homo zygotes [19].

Conversely, our study found no significant association between the TNF − 238 G/A polymorphisms and TB susceptibility at either the genotype or allele level. This finding corroborates multiple studies that have failed to establish a consistent link between this variant and TB risk. A comprehensive meta-analysis by Chen and colleagues (2020) including 15 case-control studies (3,482 cases and 4,016 controls) concluded that the TNF − 238 G/A polymorphism was not significantly associated with TB susceptibility in any genetic model [20]. The − 238 SNP, while located in the same promoter region, may have more subtle effects on gene expression or may be in the linkage disequilibrium with other functional variants that vary across populations [21].

Analysis of individual IL10 SNPs (-592 C/A, -819 C/T, -1082 G/A) revealed no statistically significant differences in genotype or allele frequencies between TB patients and controls. This finding suggests that when considered in isolation, these common promoter polymorphisms do not independently modulate TB risk in the Punjabi population. Our results align with several recent studies that have failed to demonstrate independent associations between individual IL10 SNPs and TB susceptibility. A large-scale study by Harishankar and colleagues (2020) involving 450 pulmonary TB patients and 450 healthy controls from South India reported no significant association for any of the three IL10 promoter SNPs [22]. Similarly, a meta-analysis by Tong and colleagues (2022) comprising 38 studies with 12,847 participants concluded that while the − 1082 G/A polymorphisms showed marginal significance in some ethnic subgroups, overall associations were inconsistent and population-specific [23].

The absence of individual SNP associations may be explained by the functional redundancy of these variants, their location in non-coding regulatory regions with modest individual effects on gene expression, or the presence of population-specific linkage pattern that mask main effects [24]. IL10 is a

potent anti-inflammatory cytokine that down regulates Th1 responses and macrophage activation, essential for limiting immunopathology but potentially detrimental if overexpressed during active infection [25]. The subtle effects of individual SNPs may only become apparent when considered in combination or in the context of specific environmental or microbial factors [26].

The most compelling finding emerged from haplotype analysis, which considered the combined effect of multiple SNPs within each gene. For TNF we identified four haplotypes with highly significant association. The AA and GG haplotype demonstrated strong protective effects (OR = 0.286, p = < 0.001 and OR = 0.272, p = < 0.001, respectively), while the AG and GA haplotype conferred dramatically increased TB risk (OR = 6.988, p = < 0.001 and OR = 157.181, p = < 0.001, respectively). The extraordinary odd ratio for the GA haplotype (157-fold increased risk) is particularly noteworthy and suggests that specific combination of alleles at -238 and − 308 positions create a profoundly susceptibility-enhancing genetic background.

These findings underscore the importance of haplotype-based approaches in genetic association studies. Induvial SNPs may have modest effects, but their combinations can produce synergistic functional consequences through complex interactions effecting transcription factor binding, chromatin structure, or mRNA stability [27]. Recent work by Kumar and colleagues (2022) using computational modeling demonstrated that specific TNF promoter haplotypes create distinct transcription factor binding landscapes, with the GA haplotype showing preferential binding of pro-inflammatory transcription factors including NF-κB and AP-1 [28]. This could explain the enhanced susceptibility associated with this haplotype in our population.

For IL-10, the CCA haplotype demonstrated significant protective effect (OR = 0.648; p = 0.043), while other haplotypes show no significant associations. The CCA haplotype, comprising the − 592C, -819C, and − 1082A alleles, has been previously associated with intermediate IL10 production levels [29]. In the context of TB, balanced IL10 production may be optimal- sufficient to prevent excessive tissue damage from inflammatory responses but not so high as to suppress protective Th1 immunity [30]. A recent longitudinal study by Sutherland and Colleagues (2021) found that individual carrying the CCA haplotype maintained more stable IL-10 response during *M. tuberculosis* infection and had lower rates of disease progression over five- year follow-up [31].

The distribution of genetic polymorphisms varies considerably across populations, reflecting differences in ancestral background, evolutionary selection pressures and population history [32]. Our study provides the first comprehensive analysis of these cytokine gene polymorphisms in Punjabi population of Pakistan, which represents a distinct genetic admixture of Indo-Aryan, Central Asian, and Persian influences [33]. The allele frequencies observed in our control population (TNF − 308 A: 37.2%; IL10 -1082 A: 40.4%) are broadly consistent with other South Asian populations but differ markedly from European and East Asian populations [34].

These population-specific differences have important implications for genetic association studies. A variant that confers susceptibility in one population may be neutral or protective in another due to

differences in genetic background, environmental exposures, or pathogen characteristics [35]. For example, while our study found strong associations with TNF haplotypes, a recent study from Ethiopia by Tessema and colleagues (2020) reported no significant association with either *TNF* or *IL10* polymorphisms, highlighting the importance of population-specific investigations [36].

The high prevalence of consanguineous marriages in Pakistan (estimated 60–70% in some communities) may also influence the distribution of genetic variants and the power to detect associations [37]. Our observation that genotype distributions for some SNPs deviated from Hardy-Weinberg equilibrium is consistent with this demographic characteristic and has been reported in other studies from the region [38, 39]. While such deviations can complicate genetic analysis, they also reflect the unique genetic architecture of the population and should be considered in the study design and interpretation.

To date, only limited molecular investigations of cytokine gene polymorphism have been conducted in Pakistani populations, Afzal and colleagues (2018) examined IL 10 polymorphisms in the context of hepatitis C susceptibility in Punjabi population, reporting allele frequencies similar to our control group but no significant association with infectious disease outcomes [40]. More relevant to TB, Ansari and colleagues (2019) investigated cytokines gene polymorphisms across the TB clinical spectrum in a mixed Pakistani population, finding that combinations of IFNG and IL 10 variants influenced disease severity but no initial susceptibility [41]. Our study extends these findings by demonstrating that TNF and IL10 haplotypes specifically influence susceptibility to pulmonary TB in Punjabi population, independent of disease severity or clinical presentation.

The association observed in this study have plausible biological mechanisms. TNF-alpha is essential for granuloma formation and maintenance, and its dysregulation can lead to either uncontrolled bacterial growth or excessive tissue destruction [42]. The TNF-308A allele, which formed part of our risk-associated haplotypes, has been shown in reporter gene assays to increase transcriptional activity by disrupting a repressor binding site and creating a consensus sequence for transcription factor AP-2 [43]. In the context of *M. tuberculosis* infection, this may lead to the exaggerated inflammatory responses that paradoxically impair bacterial clearance while promoting immunopathology [44].

IL10 plays a complementary role by limiting inflammation and preventing tissue damage. The protective CCA haplotype identified in our study has been associated with intermediate IL10 production in lipopolysaccharide- stimulated whole blood assays [45]. This intermediate phenotype may represent an optimal balance between adequate inflammatory responses for bacterial control and sufficient anti-inflammatory regulation to prevent tissue damage [46]. Recent work by Moreira-Teixeira and colleagues (2020) in a mouse model demonstrated that genetic ablation of IL10 led to enhanced bacterial control but increased morality from immunopathology, while IL10 over expressions resulted in uncontrolled bacterial growth [47]. These findings support the concept that optimal IL10 production, as potentially conferred by the CCA haplotype, provides the best outcome during *M. tuberculosis* infection.

This study has several strengths, including the use of GeneXpert® MTB/RIF assay confirmation for all cases, ensuring diagnostic accuracy; the inclusion of well-characterized age- and sex- matched controls from the same population; the analysis of multiple SNPs within each gene; and the comprehensive haplotype analysis that revealed strong associations not apparent from individual SNP analysis. The use of tetra-primer ARMS-PCR with Sanger sequencing confirmation provides robust genotyping data. However, the deviation from Hardy-Weinberg equilibrium for some SNPs, while consistent with population characteristics, warrants caution in interpretation and may reflect population substructure or selection biases.

Future Direction

These findings open several avenues for future investigation. Functional studies examining the effect of identified haplotypes on TNF-alpha and IL10 production in response to mycobacterial antigens would provide mechanistic validation. Larger studies incorporating genome-wide approaches could identify additional variants and gene-gene interactions contributing to TB susceptibility in this population, longitudinal studies examining the relationship between these genetic variants and treatment outcomes, disease recurrence, or development of drug resistance would have clinical relevance [48].

Conclusions

In conclusion, this study demonstrates the genetic polymorphisms in the *TNF* and *IL10* genes are significant modulators of pulmonary TB susceptibility in the Punjabi population of Pakistan. The TNF AG and GA haplotype represent major genetic risk markers, while the TNF AA/GG and IL10 CCA haplotypes provide significant protection against the disease. These findings contribute to the growing understanding of host genetics factors in TB pathogenesis and may inform future strategies for risk stratification and personalized interventions in high-burden populations. This dramatic effect sizes observed for specific haplotypes underscore the importance of comprehensive

Declarations

Ethical Approval

Advanced Studies and Research Board, University of Veterinary and Animal Sciences, Lahore Pakistan granted ethical approval of the study vide UVAS/ASRB/44 dated 01 May 2017. Written informed consent was obtained from all participants.

Conflict of Interest

The authors declare no conflicts of interest.

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Author Contribution

RMD and MYZ were responsible for the study's conceptualization, conducted material preparation, data collecting, and analysis. RMD conducted the process of collecting samples and doing laboratory testing. RMD authored the initial version of the manuscript, and all authors provided feedback on earlier iterations. MI and MYZ reviewed and edited the final manuscript. HR reviewed formatted the final version of the manuscript.

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Data Availability Statement

The original contribution presented in the study are included in the article, further inquiries can be directed to the corresponding author.

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Figures

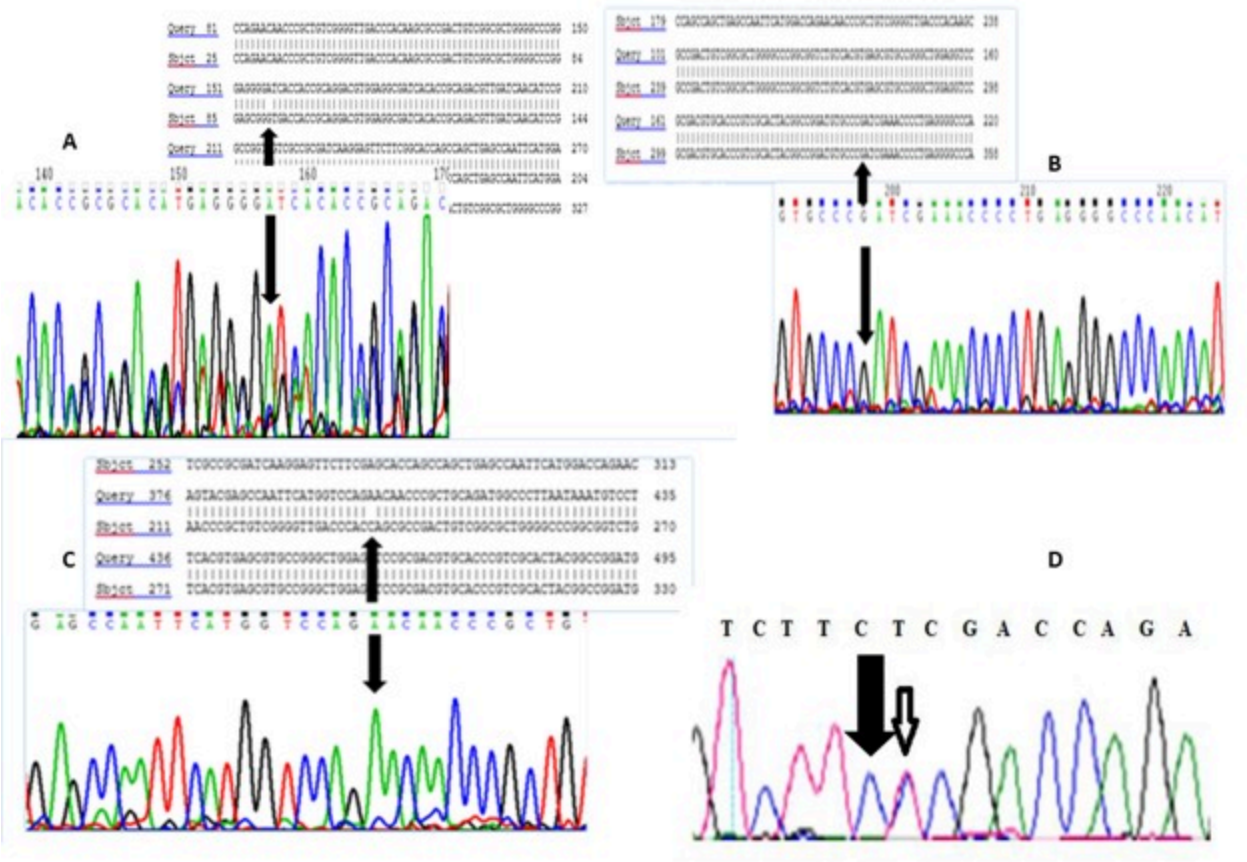


Figure 1

Chromatogram data of genes with a specific position of SNP (A) TNFA -238 A allele (amplicon position 157). (B) TNFA -308 G allele (amplicon position 198). (C) IL10 A allele (amplicon position 235). (D) IL10 Heterozygous dominant T allele

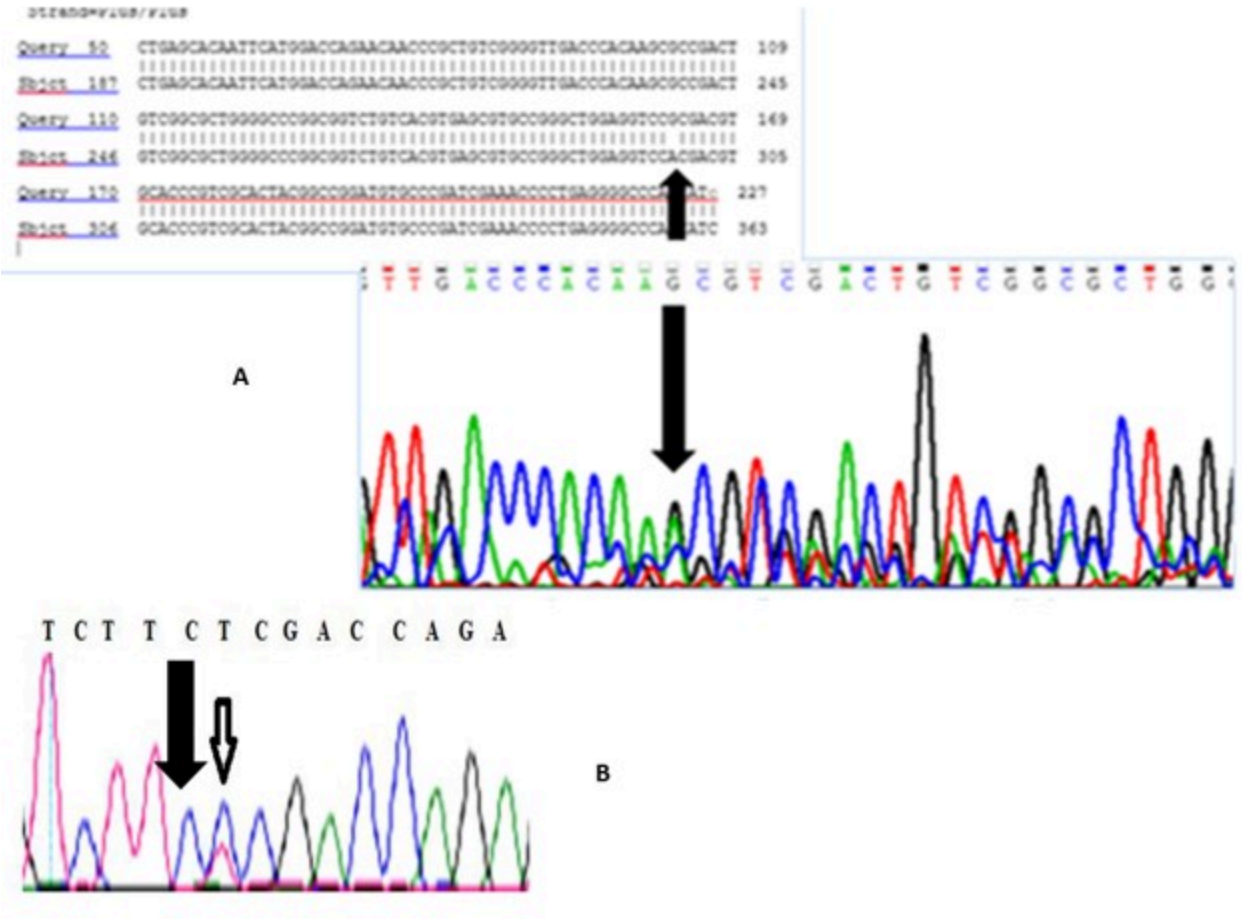


Figure 2

Chromatogram data of genes with specific position of SNP (A) IL10 Heterozygous dominant T allele. (B) G allele (amplicon position 299)

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