Monaldi Archives for Chest Disease

eISSN 2532-5264 https://www.monaldi-archives.org/

Monaldi Arch Chest Dis 2023 [Online ahead of print]

To cite this Article:
K J, Joshi JL, Kansal H, et al. **Association of *IL4RA* and *IL-8* polymorphism in predicting susceptibility towards tuberculosis.** *Monaldi Arch Chest Dis* doi: 10.4081/monaldi.2023.2594

Association of *IL4RA* and *IL-8* polymorphism in predicting susceptibility towards tuberculosis

Jyothish K,[1] Jawahar Lal Joshi,[1] Heena Kansal,[2] Vishal Chopra,[1] Kranti Garg,[1] Nidhi Girdhar,[1] Karan Sharma,[1] Rishabh Deshwal,[1] Siddharth Sharma[2]

[1]Department of Pulmonary Medicine, Government Medical College, Tuberculosis and Chest Diseases Hospital, Patiala
[2]Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, India

Corresponding Author: Siddharth Sharma, PhD, Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, India. E-mail: Siddharthsharma.phd@thapar.edu

Authors' contributions
SS, VC, KG- Data curation, Funding acquisition, Methodology, Resources, Supervision,
JK, HK- Carried out the experimental and statistical analysis
JLJ - Provides Clinical data
NG, KS, RD – Sample collection, Writing the manuscript

Competing interests: No author has a conflict of interest in this study.

Ethics approval and consent to participate: The current research work has been approved by the ethics board of Baba Farid University of Health Sciences, The approval document as issued by the Ethics Committee of Baba Farid medical college the docket number BFUHS/2K21p-TH/14858. All patients were informed about the current study and consent was taken from them.

Availability of data and materials: All data and materials related to the study shall be made available on request.

Funding: This research did not receive any specific grant from the public, commercial, or not-for-profit funding agencies.

Acknowledgements: The authors would like to express gratitude to all the subjects who participated in this study. The authors thank the Department of Chest and TB, Government Medical College, Patiala, India for providing samples and Thapar Institute of Engineering and Technology, Patiala, Punjab for providing the necessary infrastructure to carry out the research work.

Abstract

Mycobacterium tuberculosis is an infectious bacterial disease frequently affecting the lungs. With two fatalities from tuberculosis (TB) occurring every three minutes, India has the highest disease burden. The aetiology of tuberculosis has been linked to *IL-8* and *IL-4RA*. Thus, the impact of the *IL4RAQ576R* and *IL8* gene polymorphism on TB susceptibility was assessed. 301 healthy and 301 TB patients participated in a cross-sectional study. PCR RFLP was performed to identify the genotype of the IL4RAQ576R and IL-8 +781C/T gene polymorphism. The odds ratio and 95% confidence intervals were calculated using logistic regression to evaluate the risk of TB with IL4RAQ576R and IL-8 +781C/T polymorphism. A significant association was found between *IL-4RA* (p=0.04) and IL-8 +781 C/T (p= 0.03) in tuberculosis. Further, when clinical symptoms were compared with both polymorphisms, two of them, i.e., cough in *IL-4RA576R* (p=0.04) and breathlessness (p=0.01) in *IL-8 +781C/T*, showed a significant association. Moreover, different combinations of the SNPS were made, and the 3 risk allele shows a significant protective role (p=0.02). There is considerable evidence which shows that *M. tuberculosis* causes TB, an infectious disease that is genetically predisposed. The results of our study also showed that *IL-4 RA Q576R* and *IL-8 +781 C/T* played a significant protective function against tuberculosis, confirming the claim mentioned earlier. However, only the cough in *IL-4RA576R* and the dyspnea *in IL-8 +781C/T* exhibited a significant co-relation in TB patients when symptoms were examined. Additionally, the combined effects of the two SNPs were investigated, and it was discovered that the 3-risk allele has a strong association with tuberculosis. Therefore, the polymorphisms mentioned earlier, which may also be influenced by ethnicity, may significantly impact the chance of developing tuberculosis.

Key words: tuberculosis; polymorphism; *IL-8 +781 C/T*; *IL-4 RA Q576R.*

Introduction

Tuberculosis (TB) is an infectious disorder caused by *Mycobacterium tuberculosis* (Mtb) and is recognized as a significant cause of single infection source-associated mortality worldwide [1]. Several factors can cause infection risk and disease progression of TB, including malnutrition, smoking, diabetes, alcohol use, socioeconomic status, and environmental pollution [2]. Moreover, many studies have shown diverse genetic factors' vital role in host vulnerability to TB [3]. A growing number of strains are resistant to one or more anti-TB drugs constituting the leading cause for decreasing efficiency of chemotherapy, increasing number

of patients with destructive forms, increasing frequency of significant residual post-tuberculosis alterations and increasing incidence of TB relapses. Interleukin 8 (IL-8), an inflammatory cytokine of the chemokine superfamily with functional and structural correlation, was first discovered in 1987 [4]. The IL-8 gene, which spans an area of about 2.75 Mb on chromosome 4, is located in the region q13.3 and has four exons, three introns, and the proximal promoter region. It encodes a protein with 99 amino acid residues and a transcript length of 1705 base pairs [5]. The effects of IL-8 on human TB have become a particular research hotspot globally. Pathological and clinical observations have revealed prominent elevations of IL-8 levels in cerebrospinal fluid, bronchoalveolar lavage fluid, and tuberculous pleural exudate [6]. *In vivo* studies have shown that IL-8 is vital for normal immune response to TB infection and that anti-IL-8 inhibits granuloma formation.IL-8 gene regulation in monocytes and macrophages is complex, stimulus- and cell-type dependent. In most cells, it is regulated primarily at the gene transcriptional by transcriptional regulators CCAAT/enhancer binding protein beta (C/EBPβ), NF-kappaβ, AP-1 andOct-1 all having functional binding sites in IL-8 promoter [7]. The IL-8 gene has three frequent polymorphisms: *251 A/T*, *+396 G/T*, and *+781 C/T* (rs2227306). Of these, rs2227306 located on intron 1 may impact the transcriptional process because C/EBP bound preferentially in the presence of rs2227306T variation in respiratory epithelial cells [7].

IL-4Rα is a component of both the IL-4 and the IL-13 receptor complexes, which explains the similarity of the biological effect of IL-4 and IL-13 [7]. A moderate shielding effect of the variant allele at several closely related SNPs has been documented by a polymorphism in IL-4Rα. rs1801275 (A/G) of the *IL4Rα* gene in chromosome 16 (exon 12, g.54150A >G) generates a missense change (p. Gln576Arg) which effect its binding with IL-4. Thus, ending up with modulated IL-4 pathway [8]. The role of Th2 cell-mediated immunity manifested by IL-4 and IL-13 production in the pathogenesis and susceptibility remains a subject of discussion [9]. In TB, increased production of IL-4 and IL-13 is associated with lung damage, as some studies showed an increase in cavity size, indicating that signals are mediated through the common IL-4R alpha [10].

According to ALlele FREquency Database (ALFRED), where details on each population and sample are described, globally, rs2227306 has shown a frequency of 0.63 for the C allele and 0.36 for the T allele, whereas, in the case of rs1801275, A allele has a frequency of 0.77 and G allele has 0.22. Compared to the European population, frequency change has been seen for rs 2227306 (C=0.58, T=0.41), whereas no such change was seen in rs1801275 (A=0.79, G= 0.20). In the Asian population, rs2227306 has a frequency of 0.578 and 0.422 for C and T,

respectively, while 0.86 and 0.13 for C and T, respectively, in an African population. Whereas for rs1801275, it is 0.33 and 0.82 for A and 0.66, 0.16 for G in African and Asian populations, respectively.

Hence our study aimed to elucidate the role of the *IL4RA* gene and *IL-8 +781 C/T* polymorphism as a genetic marker for the risk of TB in the North Indian population. This study is the first to discover a relationship between *IL4RA* and *IL-8 +781 C/T* polymorphisms and various clinical parameters of TB patients in the same population.

Materials and Methods

Study design

A cross-sectional study was conducted. The institutional ethical committee approved the study. Three hundred and one (n=301) people were enrolled at the Department of Pulmonary Medicine, Chest and TB hospital, Government Medical College, Patiala (including 301 COPD cases and 301 healthy individuals). According to the TB diagnosis criteria, 301 cases and 301 healthy individuals were recruited. Blood samples were obtained and stored in EDTA-coated vacutainers at 20°C until DNA was isolated.

DNA extraction and genotyping

Whole blood samples from TB patients and healthy individuals were used to isolate genomic DNA using Barlett and White's method [11], which involved conventional protein K digestion, phenol/chloroform extraction, and ethanol precipitation. The presence of genomic DNA was examined on 0.8% agarose gel. The *IL-4RA* and *IL-8* polymorphisms were genotyped by polymerase chain reaction (PCR-RFLP) restriction fragment length polymorphism analysis. The target sequence was amplified by PCR using primers under a single set of reaction conditions. The PCR mixture of 15 µL comprised of 1X PCR buffer,10 X Bovine Serum Albumin, 0.5 µm forward primer, and 0.5 µm reverse primer of *IL4RAQ576R* and *IL-8 781C/T* polymorphism, 0.2 µm dNTPs and 3 U/ µL of Taq polymerase and 300ng of DNA. The primers used to amplify IL-8 781C/T variants were FP: 5'-CTCAACTCTTTATATAGGAATT -3&; RP: 5'-GATTGATTTTATCAACAGGCA -3 &; and for IL-4RA variants were: FP: 5'-GCCTTGTAACCAGCCTCTCCT - 3& RP: 5'-5-GCCCCCACCAGTGGCTACC-3. The PCR was run under the following conditions: denaturation step - 5 min at 95°C and 30 s at 94°C, annealing step – 45 s at 57°C (IL-4RA) and 42°C (IL-8), extension step – 29 cycles for 30s each at 72°C and the final extension step - 5 min at 72°C. *Msp I* (*IL-4RA*) and *EcoRI* (*IL-8 +781C/T*) enzyme was used to perform the restriction digestion of the amplicon and was

incubated at 37°C. Following digestion, the PCR products were separated on a 6% PAGE gel, and the digested bands were identified using EtBr staining. The restricted digested pattern of *IL4RAQ576R* was as follows: *AA* - wild type (107 bp and 16 bp), *AG*- heterozygous (107bp, 89bp, 18 bp and 16bp) and *GG*-mutant alleles (89bp and 18bp) (Figure 1). For IL-8, the mutant allele produced a single band of 203 bp, the wild allele produced two bands of 184 & 19 bp, as shown in Figure 2, and the heterozygous allele produced three bands of 203, 184 & 19 bp.

Statistics

The distribution of demographic attributes between cases and healthy individuals was compared using the student t-test for continuous variables and the χ^2 test for categorical data. In both cases and healthy individuals, the genotype frequencies of the *IL4RAQ576R* and *IL-8 781C/T* gene polymorphism were determined using the χ^2 test and the Hardy-Weinberg equilibrium theory (p2+2pq+q2=1), where p was the frequency of the wild-type gene, and q was the number of variant alleles. To determine whether there was a significant difference in allele and genotype frequencies between cases and healthy individuals, Pearson's χ^2 test was used. The adjusted odds ratios (AORs) and 95% confidence intervals (CI) for the risk of TB associated with the *IL4RAQ576R and IL-8 781C/T* polymorphism were calculated using logistic regression analysis with correction for potential parameters (age, smoking and gender as nominal variables) (CIs). A p-value <0.05 shall be considered to be statistically significant. For the statistical analysis, medical version 9.3.6.0 (Medcalc Software, Ostend, Belgium).

Results

Demographic and clinical characteristics among TB patients and healthy individuals

The sample population in the current study has been characterized by various demographic (i.e., age, gender, smoking status) and clinical (i.e., cough, breathlessness, fever, appetite, weight, chest pain, night sweats, hemoptysis) parameters as tabulated in Table 1. Briefly, 300 subjects for both cases and controls were recruited. Of 301 subjects, 183 were male, and 118 were females for both controls and cases, with the mean age at 34.25± 10.39 for cases and 35.7± 9.3 for controls; hence there was no significant difference between the mean ages of both cases and controls (p=0.07). No significant differences between the distribution of males and females in the case and controls were found. The number of smokers and non-smokers was compared between the two study groups. The case group comprised 19.9% smokers, whereas controls had 21.3% smokers; no significant differences were observed in the proportion of smokers and non-smokers in our study.

Overall distribution and association of *IL4RAQ576R* polymorphism with TB risk

This study evaluated the relationship between *IL4RAQ576R* polymorphism and susceptibility to TB. The allelic and genotypic distribution of *IL4RA* polymorphism, i.e., $Gln^{576}Arg$, is shown in Table 2. No significant departure was observed from Hardy-Weinberg equilibrium (HWE) for the IL-4RA genotype among cases and controls, signifying no sample bias. Statistical analysis revealed that TB patients had a lower representation of wild-type genotype (Gln/Gln), homozygous variant type (Arg/Arg) than controls (Gln/Gln: 57.48% vs 56.47%, and Arg/Arg: 3.32% vs 0.66%). In contrast, as shown in Table 2, heterozygous (Gln/ Arg) was over-represented in cases compared to control (42.85% vs 39.20%). Therefore, no significant difference was observed for genotypic distribution amongst cases and controls ($\chi 2$= 5.849, df=2, p=0.067). The minor allele frequency (MAF) for controls (0.23) was slightly higher as compared to cases (0.22). Table 2 further shows the association of TB risk with IL-4RA polymorphism. Adjusted ORs and 95% C.I. were calculated using logistic regression analysis. To further explore the correlations between the *IL-4RA* variant and TB risk, three different genetic models (codominant, dominant, and recessive) were studied. The wild genotype was considered the reference. In the codominant model, 39.20% of healthy individuals and 42.85% of cases had the heterozygous (AG) genotype. When compared to the wild-type (AA) genotype, there was no real relationship between the heterozygous (AG) genotype and risk (OR=1.11, 95% CI=0.80-1.54, p=0.52). No significant association was discovered, even after adjusting for age, gender, and smoking status (AOR=1.10, 95% CI=0.80-1.00, p=0.55). When compared to the wild genotype, the mutant (GG) genotype indicates a marginally significant reduction in the risk of developing tuberculosis (0.66% in TB patients and 3.32% in healthy individuals) (OR=0.20, 95% CI=0.04-0.94, p=0.04). The dominant model (AA vs AG+GG) did not show any relationship for TB susceptibility, as shown in Table 2. Conversely, in the recessive model, the mutant genotype has a decreased effect, showing a protective effect towards TB.

Association of *IL4RAQ576R* Polymorphism and Clinical Symptoms for TB

Based on clinical symptoms such as coughing, shortness of breath, lack of appetite, weight loss, hemoptysis, chest pain, fever, and night sweats, we also evaluated the association between *IL4RAQ576R* polymorphism and TB risk. Three models, i.e., codominant, dominant, and recessive models, were used and then adjusted with covariates like age, sex, and smoking. All the clinical symptoms were divided into two groups, whether the subject exhibited that symptom or not. For cough, In the codominant model, the mutant (*GG*) genotype was observed in 0.7% of patients having cough, and no mutant was observed in patients without cough. When

compared to the wild-type genotype (AA), the heterozygous (*GG*) genotype was found to be associated with cough in TB patients, as shown in Table 3 (OR- 2.36, 95% CI- 1.02-5.48), p=0.04). There was a 2.36-fold increase in cough in TB patients even when adjusted for cough, age, and smoking (AOR= 2.27, 95%CI= 0.97-5.33, p=0.04). Furthermore, by combining the two risk genotypes, i.e., heterozygous and mutant genotype (AG+GG) for the dominant model, we observed a 2.40-fold increased probability of cough in TB patients. Within the TB patients, breathlessness, weight loss, and appetite loss were seen in only 23.9%, 68.77% and 69.76% of patients. When the codominant and dominant model was applied to the group of TB patients, no significant results were seen, even when adjusted with covariates like age, gender, and smoking. However, TB patients having heterozygous genotypes show a protective effect against breathlessness (AOR= 0.87, 95%CI= 0.51-1.51, p=0.64), loss of appetite (AOR= 0.94, 95%CI= 0.57-1.55, p=0.79) and weight loss (AOR= 0.78, 95%CI= 0.47-1.28, p=0.33). Further, an association between IL-4RA and night sweats and chest pain was evaluated. When we compared the two using an unconditional regression model, we discovered that the IL-4RA genotype was not associated with the codominant model. When heterozygous and mutant genotypes were pooled, the dominant model also demonstrated a protective effect against night sweats and chest pain (AOR= 0.87, 95%CI= 0.43-1.77, p= 0.69) (AOR= 0.87, 95%CI= 0.43-1.77, p=0.69).

Overall distribution and association of *IL-8* polymorphism with TB risk

For this study, an association between *IL-8* polymorphism and TB was evaluated. The allelic and genotypic distribution of *IL-8 C/T* polymorphism is shown in Table 4. No significant departure was observed from Hardy-Weinberg equilibrium (HWE) for *IL-8 C/T* genotype among cases and controls, signifying no sample bias. Statistical analysis revealed that TB patients had a higher representation of the wild-type genotype (C/C) than controls (C/C: 49.5% *vs* 41.8%). In contrast, heterozygous (C/T) and mutant (T/T) were over-represented in controls as compared to cases (C/T: 49.5% *vs* 45.5%, T/T: 8.6% *vs* 4.9%). Therefore, no significant difference was observed for genotypic distribution amongst cases and controls (χ^2=5.849, df=2, p=0.067). The minor allele frequency (MAF) for controls (0.33) was higher as compared to cases (0.27). When the codominant model was applied, the heterozygous *(CT)* genotype was found in 45.5% of TB patients and 49.5% of healthy individuals. No significant association was found between the heterozygous (*CT*) genotype and TB risk (OR=0.77, 95% CI=0.56-1.08, p=0.14) when compared to the wild-type (*CC*) genotype. Even when it was adjusted with covariates such as age, gender, and smoking status, no substantial relationship was found

(AOR=0.78, p=0.16, 95% CI=0.56-1.10). The mutant (*TT*) genotype was observed in 4.96% of TB patients and 8.6% of healthy individuals. But compared with the mutant genotype, the T allele exhibits a protective effect towards TB. Similarly, when dominant (OR=0.73, p=0.06, 95% CI=0.53-1.01) and recessive (OR=0.74, p=0.06, 95% CI=0.54-1.02) models were applied, the mutant genotype had a trend exhibiting protective effects towards tuberculosis even when adjusted with age, gender, and smoking.

Association of *IL-8 781C/T* polymorphism and TB risk based on clinical symptoms

Our study assessed the association between *IL-8 C/T* polymorphism and TB risk based on clinical symptoms like coughing, shortness of breath, lack of appetite, weight loss, hemoptysis, chest pain, fever, and night sweats. When all three models, i.e., codominant, dominant, and recessive models, were used and then adjusted with covariates like age, sex, and smoking, no significant association was found in all the symptoms except breathlessness, which, when compared to wild-type genotype (*CC*) in the codominant model as shown in Table 5 (OR=1.35, 95% CI=0.78-2.39, p=0.27). Although the mutant (TT) genotype showed the risk of TB susceptibility, the association was significant (OR=0.22, 95% CI=0.06-0.75, p=0.01). Other clinical symptoms didn't show any significant results, but TB patients expressing the mutant allele have 3.66 fold and 1.092 fold of getting hemoptysis and fever, and patients carrying the heterozygous allele have 1.8 fold of having weight loss. Whereas mutant and heterozygous allele shows a borderline risk of night sweats and cough, i.e., 1.1 fold and 1.2 fold. But patients with the alleles mentioned earlier are protected from chest pain symptoms.

Combinatorial analysis of the risk associated with *IL-4RA* and *IL-8*

The genotypic combinations of the different genotypes were evaluated to see if there was a significant difference in frequency between cases and controls to evaluate SNP-to-SNP interactions. It includes individuals with heterozygous and mutant genotypes combined for the *IL-8* and *IL-4RA* genotypes polymorphism and individuals with wild genotypes for the SNPs. AOR and 95% C.I.s and the genotypic frequency of these combinations are tabulated in Supplementary Table 1. We merged the heterozygous and mutant genotypes for both SNPs into a single group. We contrasted it with the wild-type genotype, which served as the standard. (AOR=0.929, 95% CI=0.58- 1.47, p=0.75) The patients with the (H+M) genotype did not correlate significantly with tuberculosis. Further, the stratification was done based on gender to evaluate the risk of TB. However, no such association was seen for both males (AOR= 0.92, 95% CI=0.50- 1.71, p=0.80). and female (AOR=0.60, 95% CI=0.29- 1.23, p=0.16). Another

method used to study the interaction based on risk alleles was evaluating different combinations of risk alleles, as shown in Table 6. None of the alleles exhibits any risk towards TB compared to the 0-risk allele used as a reference except three risk allele, which shows a significant protective effect towards tuberculosis (AOR=0.38, 95% CI=0.16- 0.88, p=0.02) as shown in Table 6.

Discussion

Tuberculosis is a communicable disease, and despite the significant development in preventing infectious diseases, TB remains one of the most important causes of mortality. The widespread infection over time indicates powerful evolutionary pressures in the interaction between host and pathogen genomes. This susceptibility to tuberculosis and host genetic factors have been studied extensively using case-control, candidate genes and genome-wide linkage methods. Many essential candidate genes like HLA genes, on HLA genes such as cytokines and their receptors, chemokines and their receptors, pattern recognition receptors-TLRs, mannose-binding lectin and the dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin), solute carrier family 11A member1 and purinergicP2X7 receptor gene polymorphisms have been associated with differential susceptibility to TB in various ethnic populations. The current study determined the SNPs with IL-4, *IL-4Ralpha* and *IL-8* and their susceptibility to pulmonary tuberculosis.

***IL-4RAQ576R* polymorphism and its relationship with tuberculosis**

Previous studies suggest that viral or mycobacterial infection may suppress IgE levels by inhibiting a Th2 immune response. The fact that *Mycobacterium tuberculosis* infection causes a type-1 immune response has been substantiated by a study. Directly in opposition, intestinal parasites cause a type-2 reaction, and these two reactions -type-1 and type-2- inhibit each other and subsequently lower serum IgE, an indication of a type-2 reaction [12]. A cytokine of the Th2 subtype, IL-4, can promote the Th2 immune response. Numerous studies have shown that the rs1801275 G allele, or the *IL-4R* allele, is associated with higher levels of IL-4R when arginine is present at position 576 in place of glutamine. This is because rs1801275 (A/G) is a gain of function mutation that accelerates signal transduction. After all, it is located next to the crucially important tyrosine-2 residue, which is a component of the docking site for STAT6, and that's why it can predominate Th2 immune response pattern [13]. Several diseases, including rheumatoid arthritis [14], atopic problems [15], asthma, and many others, have been linked to polymorphic variants of the IL4RA gene [16-19].

To our knowledge, however, only a small number of research have connected the *IL4RAQ576R* polymorphism to tuberculosis. Hence, this study evaluated the relationship between *IL-4RAQ576R* with TB patients. When all three models were applied, it was observed that in the codominant model, when the mutant allele was compared with the wild type, a significant association was seen, and the mutant allele, i.e., IL-4RA 576R showed a significant protective role towards tuberculosis. The notion above is supported by the fact that *IL-4RA 576R* demonstrates a considerable protective role in the recessive model. Also, previous studies have hypothesized that TB is not due to lack of Th1 but due to decreased Th2-like response, including interleukin-4 (IL-4), and our results have also supported this fact as rs1801275 modulates the binding of IL-4 with its receptor which is a major contributor to Th2 like a response [20].

Relation of *IL-4RAQ576R* polymorphism in association with clinical symptoms

We also investigated the role of the *IL-4RAQ576R* polymorphism in clinical symptoms such as cough, breathlessness, appetite loss, weight loss, chest pain, hemoptysis, fever, and night sweats. To the best of our knowledge, this is the first study illustrating the contribution of *IL4RAQ576R* polymorphisms to symptoms in tuberculosis. The codominant model showed a substantial correlation between cough and the heterozygous allele compared to the wild type. The link was substantial when the dominant model was used, with a 2.40-fold increased likelihood of cough in TB patients. Similarly, no significant results were found when codominant and dominant models were applied to breathlessness, lack of appetite, weight loss, and chest pain, but all of these symptoms had marginally protective effects towards these symptoms in TB patients. However, when the same models were applied to hemoptysis and fever, a somewhat increased risk for these symptoms was seen, but no significant results were discovered.

***IL-8 +781C/T* polymorphism and its relationship with tuberculosis**

Leukocytes are attracted to inflammatory areas by IL-8. Leukocytes produce and release it in response to M. tuberculosis or its constituent parts. The IL-8 found on chromosome 4q13- 21 consists of four exons, three introns, and the proximal promoter. Several polymorphisms have been discovered in the IL-8 gene. An increased cytokine IL-8 has been linked to a polymorphism (rs2227306) in the promoter region (Intron 1) of the gene for IL-8, which is 781C/T. The T allele is coupled to a preference for C/EBP-b binding in lung epithelial cells. C/EBP-b binds the IL-8 gene's regulatory region, a crucial inflammation regulator that

significantly boosts transcription [20-22]. Many studies have linked various SNPs to TB, even in interleukin-8 [23,24], but very few have investigated rs2227306 with tuberculosis. Our study investigated the relationship between *IL-8 +781C/T* polymorphism with tuberculosis. Our research revealed a significant association between the mutant allele T and the wild-type C allele, indicating that the T allele of interleukin-8 at the +781 location has a protective effect on tuberculosis (TB) (OR=0.49, p=0.03, 95% CI=0.24- 0.96). Many studies have demonstrated that the C allele increases the production of IL-8 and the susceptibility to the disease. The highly produced IL-8 may encourage polymorphonuclear leukocyte apoptosis, and then induce inflammation. Additionally, the increased expression of IL-8 draws an excessive number of leukocytes to the lesion site, where they produce proteases, elastases and free radicals that significantly damage the tissue.

Relation of *IL-8 +781C/T* polymorphism in association with clinical symptoms

We have also correlated all the symptoms discussed earlier to *IL-8 +781C/T*. All three models were applied, and instead of breathlessness, none of the symptoms exhibited a significant correlation with *IL-8 +781C/T* in TB patients. The T allele shows a protective effect towards symptoms like breathlessness, loss of appetite, and chest pain in TB patients. But the heterozygous allele, compared to the wild type in codominant, shows the risk of appetite loss and breathlessness in TB patients. In comparison, the T allele has been associated with an increased risk of cough in TB patients in codominant (1.80-fold), dominant (1.26-fold), and recessive (1.64) models. When the codominant model was applied to evaluate the co-relation of risk of loss of weight with *IL-8 +781C/T*, it was found that the heterozygous genotype shows a borderline protective effect.

In contrast, the T allele increases the risk of weight loss by 1.73-fold and 1.86-fold in the recessive model. Also, the T allele has been shown to increase hemoptysis by 3.66-fold using the codominant model and 2.77-fold in the recessive model, whereas wild type and heterozygous decrease these risks compared to mutant type. Fever and night sweats also enhanced, as the T allele increased these symptoms by 1.92 and 1.11-fold when the codominant model was applied.

Combinatorial analysis of the risk associated with rs1801275 and rs2227306

We have employed two methods in our study to evaluate the collective risk of these SNP towards TB by employing different combinations possible for both. Many studies have investigated the association between various SNPs [25], but till now, no such association has

been evaluated in tuberculosis for these. When heterozygous and mutant genotypes common in both the SNPs were compared with the wild type, no significant associations were found, even when applied separately to males and females. But all of them show a protective effect; even in females, the odds were slightly less than in males. In another method, various combinations of the risk allele were made and compared to the 0-risk allele, i.e., wild type of both the SNPs, but except for the three-risk allele, which shows a significant association, none shows a significant association. But it is interesting to note that as the number of risk allele increase, the protective effect increases, and the 3-risk allele shows a significant outcome.

Limitations of the study

It is essential to mention that the present study has certain limitations. One of the key impediments to our research is the small sample size. Our findings regarding the association of *IL4RAQ576R and IL-8 +781 C/T* risk were restricted to the North Indian population. Due to ethnic or regional differences, the conclusions may vary in different countries or regions. A thorough analysis of extended haplotypes across candidate genes must also be combined with functional studies of gene and protein function to determine which polymorphisms are disease-associated. Therefore, to confirm the findings, large, extensive case-control studies involving people of different nationalities and ethnicities are precious for understanding how TB develops and may aid in early disease prevention.

Strengths of the study

On the other hand, the present study has certain advantages. To the best of our knowledge, this is the first study illustrating the contribution of *IL4RAQ576R, IL-8 C/T* TB polymorphisms to TB in the North Indian population. Our study is the first to assess and report the stratifications between the clinical indicators pertaining to TB and *IL4RAQ576R, IL-8 C/T* polymorphism. Additionally, we have used explicit criteria for study inclusion and a strict procedure for data extraction.

Conclusions

In our study, *IL-4 RA 576R* and *IL-8 +781 C/T* showed a significant protective role towards tuberculosis. Whereas when symptoms was evaluated, TB patients show significant association, only towards cough in *IL-4RA576R* and breathlessness in *IL-8 +781C/T*. Further on combined effects of both the SNPs were evaluated and 3 risk allele shows a significant association with TB.

References

1. Dara M, Acosta CD, Melchers NV, et al. Tuberculosis control in prisons: current situation and research gaps. Int J Infect Diss 2015 1;32:111-7.
2. Bloom BR, Small PM. The evolving relation between humans and Mycobacterium tuberculosis. N Engl J Med 1998;338:677-8.
3. Antonucci G, Girardi E, Raviglione MC, et al. Risk factors for tuberculosis in HIV-lnfected persons: a prospective cohort study. JAMA 1995;274:143-8.
4. Chakrabartty A, Basu P, Ali KM, et al. Tuberculosis related stigma and its effect on the delay for sputum examination under the Revised National Tuberculosis Control Program in India. Indian J Tuberc 2018;65:145-51.
5. Mukaida NA, Shiroo MA, Matsushima KO. Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. J Immunol 1989;143:1366-71.
6. Agarwal R, Srinivas R, Aggarwal AN, Saxena AK. Pulmonary masses in allergic bronchopulmonary aspergillosis: mechanistic explanations. Respir Care 2008;53:1744-8.
7. Wani RL. Tuberculosis 2: Pathophysiology and microbiology of pulmonary tuberculosis. South Sudan Med J 2013;6:10-2.
8. North RJ, Jung YJ. Immunity to tuberculosis Annu Rev Immunol 2004;22:599-623.
9. Jimenez-Sousa MA, López E, Fernandez-Rodríguez A, et al. Genetic polymorphisms located in genes related to immune and inflammatory processes are associated with end-stage renal disease: a preliminary study. BMC Med Genet 2012;13:58.
10. van Crevel R, Ottenhoff TH, van der Meer JW. Innate immunity to Mycobacterium tuberculosis. Clin Microbiol Rev 2002;15:294-309.
11. Bartlett JM, Stirling D. PCR protocols. Totowa, NJ: Humana Press; 2003.
12. Ohrui T, Zayasu K, Sato E, et al. Pulmonary tuberculosis and serum IgE. Clin Exp Immunol 2000;122:13-5.
13. Hershey GK, Friedrich MF, Esswein LA, et al. The association of atopy with a gain-of-function mutation in the α subunit of the interleukin-4 receptor. N Engl J Med 1997;337:1720-5.
14. Moreno O, González CI, Saaibi DL, et al. Polymorphisms in the IL4 and IL4RA genes in Colombian patients with rheumatoid arthritis. J Rheumatol 2007;34:36-42.
15. Howard TD, Koppelman GH, Xu J, et al. Gene-gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma. Am J Hum Gen 2002;70:230-6.
16. Hegab AE, Sakamoto T, Saitoh W, et al. Polymorphisms of IL4, IL13, and ADRB2 genes in COPD. Chest 2004;126:1832-9.
17. Li L, Li Y, Zeng X, et al. Role of interleukin-4 genetic polymorphisms and environmental factors in the risk of asthma in children. Genet Mol Res 2016;15:534-43.
18. Beghé B, Barton S, Rorke S, et al. Polymorphisms in the interleukin-4 and interleukin-4 receptor α chain genes confer susceptibility to asthma and atopy in a Caucasian population. Clin Exp Allergy 2003;33:1111-7.
19. Rosa-Rosa L, Zimmermann N, Bernstein JA, et al. The R576 IL-4 receptor α allele correlates with asthma severity. J Allergy Clin Immunol 1999;104:1008-14.
20. Hafez SF, Amer OT, Mostafa OA, Samy WM. Polymorphism RS2227306 in the interleukin 8 gene and its relation to bronchial asthma. Egypt J Hospital Med 2021;86:50-

3.
21. Rook GA, Hernandez-Pando R, Dheda K, Seah GT. IL-4 in tuberculosis: implications for vaccine design. Trends Immunol 2004;25:483-8.
22. Ma X, Reich RA, Wright JA, et al. Association between interleukin-8 gene alleles and human susceptibility to tuberculosis disease. J Infect Dis 2003;188:349-55.
23. Krupa A, Fol M, Dziadek BR, et al. Binding of CXCL8/IL-8 to Mycobacterium tuberculosis modulates the innate immune response. Mediators Inflamm 2015;2015:124762.
24. Selvaraj P, Anand SP, Jawahar MS, et al. Promoter polymorphism of IL-8 gene and IL-8 production in pulmonary tuberculosis. Curr Sci 2006:952-4.
25. Chen J, Ma A. Associations of polymorphisms in interleukins with tuberculosis: Evidence from a meta-analysis. Immunol Lett 2020;217:1-6.
26. Walia HK, Sharma P, Singh N, Sharma S. Synergistic polymorphic interactions of phase II metabolizing genes and their association toward lung cancer susceptibility in North Indians. Int J Environ Health Res 2022. Online ahead of print.

Table 1. Demographic characteristics among cases and controls.

Variable	Total (N)	Cases, n (%)	Controls, n (%)	p-value*
Age (years)	301			
Mean ± SD		34.25 ± 10.39	35.7 ± 9.3	0.07
Range		18-50	15-50	
Gender	301			
Male		183 (60.8)	183 (60.8)	
Female		118 (39.2)	118 (39.2)	1.00
Smoking status	301			
Smokers		60 (19.9)	64 (21.3)	
Non-smokers		241 (80.1)	237 (78.7)	0.68

*p-values were derived from Pearson χ^2test except for age and pack-years; Student's *t*-test was used for age and pack-years; sll p-values are two-sided; $p<0.05$ was considered statistically significant.

Table 2. Genotypic and allelic distribution of *IL-4RA* genetic variant and its association with risk of tuberculosis.

Polymorphism *IL4RAQ576R*	**Controls**	**Cases**				
Codominant	**n (%) N=301**	**n (%) N=301**	**OR[*] (95% CI)**	**p[#]**	**AOR[°] (95% CI)**	**p[§]**
AA	173 (57.48)	170 (56.47)	1.00 (Reference)	-	1.00 (Reference)	-
AG	118 (39.20)	129 (42.85)	1.11 (0.80-1.54)	0.52	1.10 (0.80-1.00)	0.55
GG	10 (3.32)	2 (0.66)	0.20 (0.04-0.94)	**0.04**	0.20 (0.04-0.94)	**0.04**
Gln (allele)	464	469				
Arg (allele)	138	133				
MAF	0.23	0.22				
		χ^2= 5.849, df=2, p=0.067				
Dominant	**n (%) N=301**	**n (%) N=301**	**OR[*] (95% CI)**	**p[#]**	**AOR[°] (95% CI)**	**p[§]**
AA	173 (57.48)	170 (56.4)	1.00 (Reference)	-	1.00 (Reference)	-
AG+GG	128 (42.52)	131 (43.5)	1.04 (0.75-1.43)	0.80	1.03 (0.75-1.43)	0.83
Recessive	**n (%) N=301**	**n (%) N=301**	**OR[*] (95% CI)**	**p[#]**	**AOR[°] (95% CI)**	**p[§]**
AA+AG	291 (96.68)	299 (99.3)	1.00 (Reference)	-	1.00 (Reference)	-
GG	10 (3.32)	2 (0.66)	0.19 (0.042-0.90)	**0.04**	0.19 (0.04-0.90)	**0.03**

*Odds ratio, 95% confidence intervals; [#]corresponding p-values were calculated by logistic regression analysis; °adjusted odds ratio, 95% confidence interval calculated; [§]p-value after being adjusted.

Table 3. Association of *IL-4RA* and cough for tuberculosis.

Polymorphism IL4RAQ576R	**With cough**	**Without cough**	**Cases**			
Codominant	**n (%) N=270**	**n (%) N= 31**	**OR[*] (95% CI)**	**p[#]**	**AOR[°] (95% CI)**	**p[§]**
AA	147(54.44)	8 (25.80)	1.00 (Reference)	-	1.00 (Reference)	-
AG	121 (44.81)	23 (74.19)	2.36 (1.02-5.48)	**0.04**	2.27 (0.97-5.33)	0.04
Dominant	**n (%) N=270**	**n (%) N=31**	**OR[1] (95% CI)**	**p[#]**	**AOR[°] (95% CI)**	**p[§]**
AA	147 (54.44)	23 (74.19)	1.00 (Reference)	-	1.00 (Reference)	-
AG+GG	123 (45.55)	8 (25.80)	2.40 (1.04-5.57)	**0.04**	2.30 (0.98-5.40)	0.04

*Odds ratio, 95% confidence intervals; [#]corresponding p-values were calculated by logistic regression analysis; °adjusted odds ratio, 95% confidence interval calculated; [§]p-value after being adjusted.

Table 4. Genotypic and allelic distribution of *IL-8* genetic variant and its association with risk of tuberculosis.

Polymorphism *IL-8*	**Controls**	**Cases**				
Codominant	**n (%) N=301**	**n (%) N=301**	**OR[*] (95% CI)**	**p[#]**	**AOR[°] (95% CI)**	**p[§]**
CC	126 (41.8)	149 (49.5)	1.00 (Reference)	-	1.00 (Reference)	-
CT	149 (49.5)	137 (45.5)	0.77 (0.56-1.08)	0.14	0.78 (0.56-1.10)	0.16
TT	26 (8.6)	15 (4.9)	0.49 (0.24-0.96)	**0.03**	0.50 (0.25-0.99)	**0.04**
C (allele)	401	435				
T (allele)	201	167				
MAF	0.33	0.27				
		$\chi 2$= 5.849, df=2, p= 0.067				
Dominant	**n (%) N=301**	**n (%) N=301**	**OR[1] (95% CI)**	**p[#]**	**AOR[2](95% CI)**	**p[§]**
CC	126 (41.8)	149 (49.5)	1.00 (Reference)	-	1.00 (Reference)	-
CT+TT	175 (58.1)	152 (50.4)	0.73 (0.53-1.01)	0.06	0.74 (0.54-1.02)	0.06
Recessive	**n (%) N=301**	**n (%) N=301**	**OR[1] (95% CI)**	**p[#]**	**AOR[2](95% CI)**	**p[§]**
CC+CT	275 (91.3)	286 (95.01)	1.00 (Reference)	-	1.00 (Reference)	-
TT	26 (3.32)	15 (3.96)	0.55 (0.28-1.06)	0.07	0.56 (0.28-1.08)	0.06

*Odds ratio, 95% confidence intervals; [#]corresponding p-values were calculated by logistic regression analysis; °adjusted odds ratio, 95% confidence interval calculated; [§]p-value after being adjusted.

Table 5. Association of *IL-8* and breathlessness for tuberculosis.

Polymorphism IL-8	**With** breathlessness	**Without** breathlessness				
Codominant	**n (%) N=72**	**n (%) N=229**	**OR[*] (95% CI)**	**p[#]**	**AOR[°] (95% CI)**	**p[§]**
CC	32 (57.48)	117 (61.88)	1.00 (Reference)	-	1.00 (Reference)	-
CT	37 (39.20)	100 (34.15)	1.35 (0.78-2.39)	0.27	1.39 (0.80-2.42)	0.22
TT	3 (3.32)	12 (3.96)	0.22 (0.06-0.75)	**0.01**	0.21 (0.06-0.74)	**0.01**
Dominant	**n (%) N=72**	**n (%) N=229**	**OR[1] (95% CI)**	**p[#]**	**AOR[2](95% CI)**	**p[§]**
CC	32 (41.8)	117 (49.5)	1.00 (Reference)	-	1.00 (Reference)	-
CT+TT	40 (58.1)	112 (50.4)	0.22 (0.06-0.75)	**0.01**	0.21 (0.06-0.74)	**0.01**
Recessive	**n (%) N=72**	**n (%) N=229**	**OR[1] (95% CI)**	**p[#]**	**AOR[2](95% CI)**	**p[§]**
CC+CT	69 (91.3)	217 (95.01)	1.00 (Reference)	-	1.00 (Reference)	-
TT	3 (3.32)	12 (3.96)	0.78 (0.21-2.86)	0.71	0.80 (0.21-2.97)	0.74

*Odds ratio, 95% confidence intervals; #corresponding p-values were calculated by logistic regression analysis; °adjusted odds ratio, 95% confidence interval calculated; §p-value after being adjusted.

Table 6. Combined effects of *IL-4RA* (A/G) and *IL-8* (C/T) on the risk of tuberculosis.

No. of risk allele	Controls	Cases	OR*	p#	AOR° (95% CI)	p§
0	82	86	1.00 (Reference)	-	1.00 (Reference)	-
1	121	139	1.09	0.6459	1.21 (0.77-1.89)	0.71
2	76	67	0.84	0.4459	0.85(0.54-1.33)	0.48
3	22	9	0.39	**0.0266**	0.38(0.16-0.88)	**0.02**

*Odds ratio, 95% confidence intervals; #corresponding p-values were calculated by logistic regression analysis; °adjusted odds ratio, 95% confidence interval calculated; §p-value after being adjusted; 0, (C/C, A/A); 1, (C/C, A/G) (C/T, A/A); 2, (C/T, A/G) (T/T, A/A), (C/C, G/G); 3, (C/T, GG) (T/T, A/G).

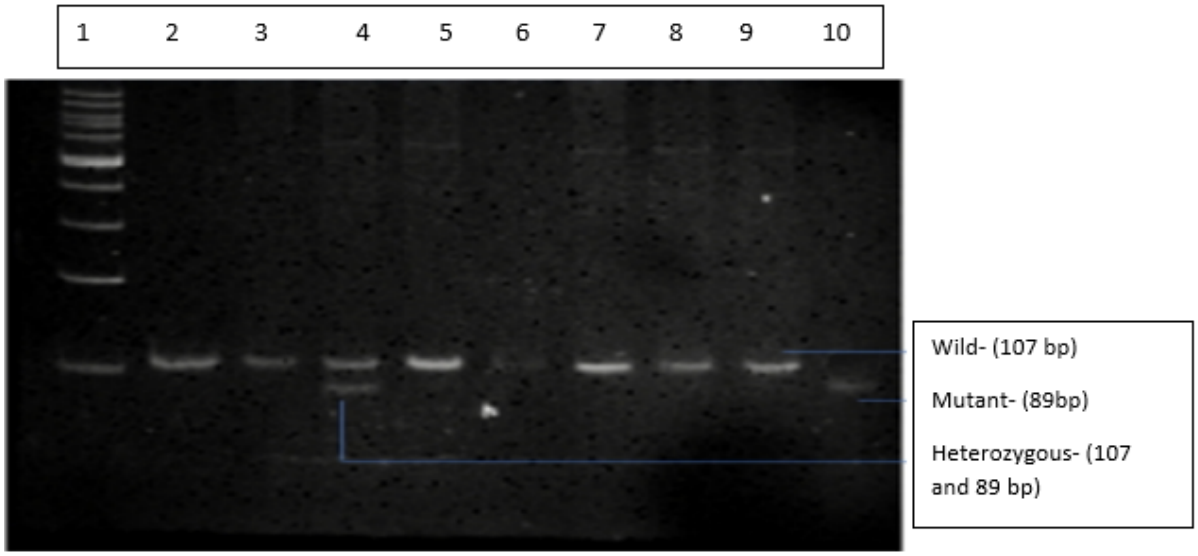

Figure 1. 6% PAGE gel of digested PCR for the detection of genotypes of *IL-4RA* 576R polymorphism.

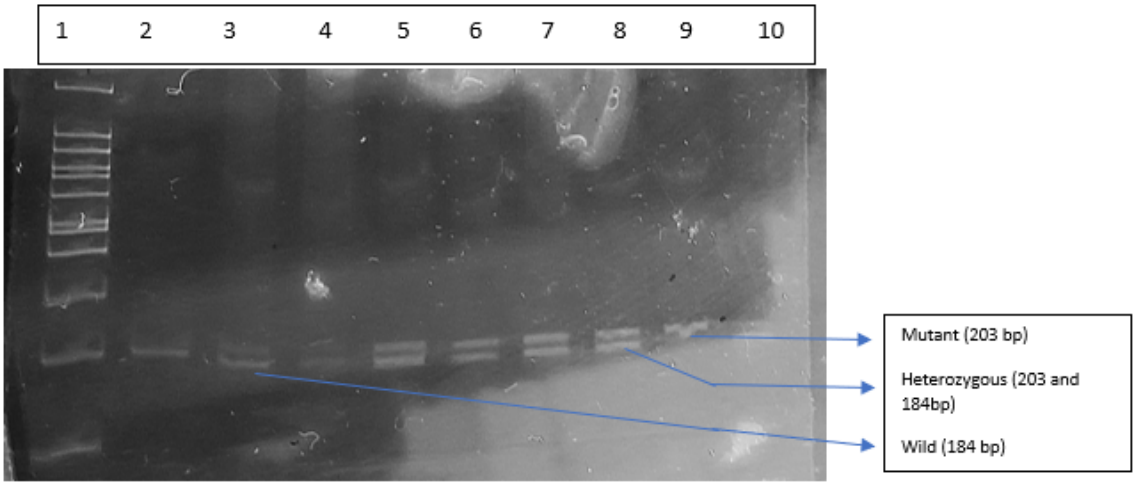

Figure 2. 6% PAGE gel of digested PCR for the detection of genotypes of *IL-8* 781C/T polymorphism.