

# Lack of Association between Interleukin-18 –607 C/A Gene Polymorphism and Pulmonary Tuberculosis in Zahedan, Southeast Iran

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**Abstract:** Interleukin-18 (IL-18) plays a critical role in immune response, contributing to the pathogenesis and pathophysiology of infectious diseases. Polymorphisms in the IL-18 genes are known to influence expression levels and may be associated with outcome of infections. The objective of this study was to determine whether the presence of IL-18 polymorphisms –607 A/C (rs1946518) was associated with tuberculosis disease. We investigated the functional polymorphism of IL-18 (rs1946518) in 174 patients with pulmonary tuberculosis (PTB) and 177 healthy subjects. Genotype analysis was done using tetra amplification refractory mutation system-PCR (T-ARMS-PCR). The allelic and genotypic frequencies of the IL-18 polymorphism did not differ significantly between PTB and the controls. Our finding suggests that IL-18 polymorphism (rs1946518) may not be a risk factor for susceptibility to tuberculosis in a sample of Iranian population. Further studies are required to validate our findings.

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## Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a major cause of morbidity and mortality throughout the world especially in Asia and Africa and remains a public health problem. It can affect almost all of the body, including the brain, the kidneys and the bones, but predominately manifests itself in the lungs where it is called pulmonary tuberculosis.

One third of the earth's population is infected with TB but only 10% individuals infected will develop the disease and about 2 million people die due to the disease each year. Increasing evidence indicates that host genetic factors play an important role in susceptibility to TB (Stein, 2011).

IL-18 is a proinflammatory cytokine produced mainly by macrophages and monocyte but also by dendritic cells, Kupffer cells, keratinocytes, synovial, fibroblasts, epithelial cells, and osteoblasts cells and plays a crucial role in regulation of both innate and acquired immunity and have an important role in inflammatory and infectious diseases (Schneider et al., 2010).

IL-18, in synergy with IL-12 or IL-21 enhances IFN- $\gamma$  production in human NK and T Cells (Shigehara et al., 2001; Strengell et al., 2003). The IL-18 gene is located on chromosome 11(11q22.2–22.3), and contains many polymorphism, especially in the promoter region (Smith and Humphries, 2009). There are two polymorphisms (–137 G/C and –607 A/C) in promoter seems to affect the expression level of IL-18 at transcription level (Giedraitis et al., 2001). To the best of our knowledge, there are only two reports regarding the association of IL-18 gene polymorphisms and pulmonary tuberculosis (Harishankar et al., 2007; Han et al., 2011). The present study aimed to determine the possible association between IL-18 –607 C/A gene polymorphism and pulmonary tuberculosis in a sample of Iranian population.

## Material and Methods

This case-control study was performed on 174 patients with pulmonary tuberculosis (110 women and 64 men; ages  $50.17 \pm 20.47$ ) and 177 healthy subjects (97 women and 80 men; ages  $46.88 \pm 15.45$ ). There was no significant difference among the groups regarding sex and age ( $p > 0.05$ ). The diagnosis of PTB was based on clinical, radiological, sputum Acid Fast Bacillus (AFB) smear positivity, culture and response to antituberculosis chemotherapy as described previously (Naderi et al., 2009, 2010).

The project was approved by the local Ethics Committee of the Zahedan University of Medical Sciences, and written informed consent was taken from all participants. Blood samples were collected in Na-EDTA tubes from patients and healthy controls and stored at  $-20\text{ }^{\circ}\text{C}$  until DNA extraction.

Genomic DNA extraction from blood samples was carried out as described previously (Hashemi et al., 2010a). Tetra primer ARMS-PCR is a simple and rapid method for detection of single nucleotide polymorphism (SNP) (Ye et al., 2001; Hashemi et al., 2010b, 2011a; Naderi et al., 2011). We designed tetra-ARMS-PCR

for detection of –607 C/A polymorphism of IL-18 gene. We used two external primers (forward outer and reverse outer) and two inner primers (forward inner and reverse inner) that are shown in Table 1.

Product sizes were; 208 bp for C allele and 278 bp for A allele, while the product size of the two outer primers (control band) was 440 bp as is shown schematically in Figure 1.

Polymerase chain reaction (PCR) was performed using commercially available PCR premix (AccuPower PCR PreMix, BIONEER, Daejeon, South Korea) according to the manufacturer recommended protocol. Into a 0.2-ml PCR tube containing the AccuPower PCR Pre-Mix, 1 µl template DNA (~100 ng/µl), 1 µl of each primer (10 µM) and 15 µl DNase-free water were added. The total volume for the PCR was 20 µl.

The PCR protocol for detection of –607 C/A polymorphism of IL-18 was as follows: initial denaturation at 95 °C for 5 min followed by 30 cycles, consisting of denaturation at 95 °C for 30 s, annealing at 54 °C for 20 s, and extension at 72 °C for 30 s. Final extension was performed at 72 °C for 10 min. The PCR products were analyzed by electrophoresis on a 2% agarose gel containing 0.5 µg/ml ethidium bromide and visualized by transillumination with UV light and photograph was taken (Figure 2). We re-genotyped approximately 10% of the samples to verify the initial results. The check confirmed the previous genotyping results by 100%.

The statistical analysis of the data was performed using the SPSS 18.0 software. Demographics and biochemical parameters between the groups were analysed

**Table 1 – Primers used for polymorphism determination**

Primer	Sequence (5'→3')	T <sub>m</sub> (°C)
Forward outer primer	CCTACAATGTTACAACACTTAAAAT	50
Reverse outer primer	ATAAGCCCTAAATATATGTATCCTTA	49
Forward inner primer	GATACCATCATTAGAATTTTGTG	48
Reverse inner primer	GCAGAAAGTGAAAAATTATCAA	48

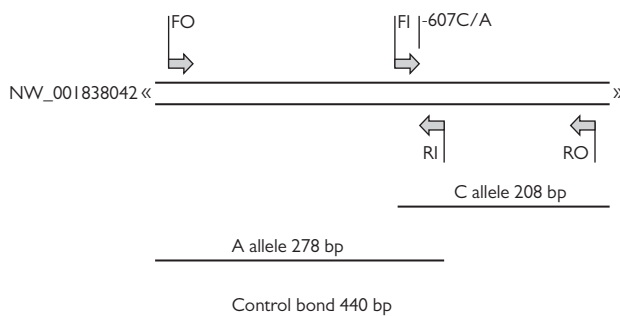


Figure 1 – Schematic illustration of tetra amplification refractory mutation system-polymerase chain reaction (tetra-ARMS-PCR) assay for detection of IL-18 –607 C/A polymorphism. Two forward and two reverse specific primers are used to produce three potential products. Product sizes were: 208 bp for C allele, 278 bp for A allele and 440 bp for two outer primers (control band).

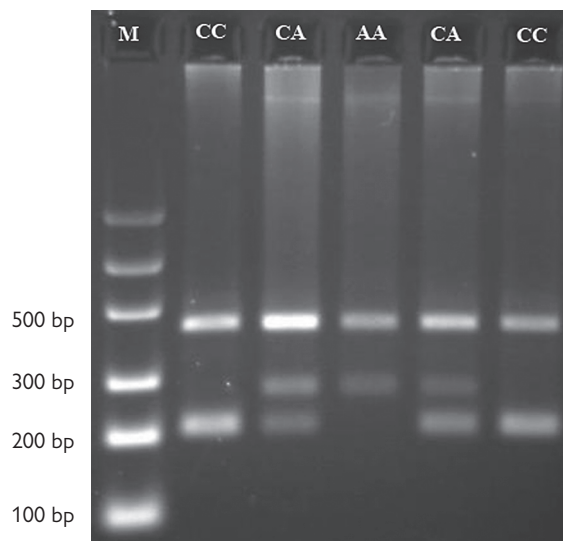


Figure 2 – Electrophoresis pattern of tetra amplification refractory mutation system-polymerase chain reaction (tetra-ARMS-PCR) for detection of IL-18 –607 C/A polymorphism.

by independent sample *t*-test for continuous data and  $\chi^2$  test for categorical data. The associations between genotypes of IL-18 gene and PTB were estimated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses.

## Results

The allele and genotype frequencies in patient and control groups were summarized in Table 2. The wild-type genotype (CC) was observed in 74 (42.5%) of the patients; whereas 80 (46.0%) were heterozygous (CA) and 20 (11.5%) were homozygous (AA). In the control group, the frequencies of genotypes were 68 (38.5%) for CC, 90 (50.8%) for CA and 19 (10.7%) for AA. There were no significant differences regarding –607 C/A polymorphism of IL-18 among PTB patients and normal

**Table 2 – The genotypes and allele distribution of IL-18 polymorphism in case and control groups**

Polymorphism	PTB n (%)	Normal n (%)	OR (95% CI)	p	*OR (95% CI)	p
<i>Genotypes</i>						
CC	74 (42.5)	68 (38.5)	1.00		1.00	
CA	80 (46.0)	90 (50.8)	0.82 (0.52–1.28)	0.374	0.79 (0.50–1.24)	0.307
AA	20 (11.5)	19 (10.7)	0.97 (0.48–1.97)	0.927	1.00 (0.49–2.04)	0.995
<i>Alleles</i>						
C	228 (65.5)	226 (63.8)				
A	120 (34.5)	128 (36.2)	0.93 (0.68–1.27)	0.693		

\*adjusted for age and sex

subjects ( $p=0.656$ ). No significant differences were observed among PTB and control subjects regarding allele frequencies (0.693).

The IL-18 –607 C/A genotypes polymorphism in case and control groups was consistent with Hardy-Weinberg equilibrium.

## Discussion

Tuberculosis kills more people each year than any other single infectious disease. Development of tuberculosis disease depends on interaction between the pathogen, environment, and the host. Many studies support that the differences in host immune genes is accompanying with susceptibility/resistance to TB (Sharma et al., 2010; Hashemi et al., 2011b).

IL-18 which is an important interferon- $\gamma$  (IFN- $\gamma$ ) inducer can activate immune cells, promoting the activation and proliferation of T and natural killer cells and the antituberculosis response (Sugawara, 2000). IL-18 is involved in protective immunity against a broad range of intracellular pathogens. It has been reported that mice lacking IL-18 were highly susceptible to *M. tuberculosis* infection and succumbed more rapidly than WT B6 mice (Schneider et al., 2010).

It is being thought that two SNPs in the promoter region of the IL-18 gene regulate the expression of gene at the transcriptional level and changed the production level of IL-18 (Giedraitis et al., 2001). Indeed variation at –607 C/A of IL-18 gene disrupts cAMP-responsive element-binding protein binding site, influence the quantity of transcribed IL-18 mRNA (Giedraitis et al., 2001). Khripko et al. (2008) demonstrated that subjects carrying allele –607 A have significant increase in IL-18 production compared to wild type in Southwestern Siberia. IL-18, in synergy with IL-12 or IL-21 enhances IFN- $\gamma$  production in human NK and T Cells (Shigehara et al., 2001; Strengell et al., 2003). In our previous study, we showed that IFN- $\gamma$  +874 T/A polymorphism confers susceptibility to pulmonary tuberculosis in a sample of Iranian population (Hashemi et al., 2011b). In the present study, we attempted to determine the impact of IL-18 –607 C/A polymorphism on the susceptibility of PTB in our population. We found no association between IL-18 –607 C/A polymorphism and susceptibility to PTB. Our results are in agreement with the findings of Harishankar et al. (2007). They found that the IL-18 gene promoter polymorphisms are not associated with susceptibility or resistance to pulmonary tuberculosis in south Indian population. Contrary to our findings, Han et al. (2011) demonstrated an association between IL-18 polymorphisms and susceptibility to PTB and increase risk of developing the disease in Chinese Han population. The controversy results suggesting that the impact of IL-18 –607 C/A gene polymorphism on PTB is likely to be influenced by ethnic origin.

Many studies demonstrated productivity role of IL-18 in *Mycobacterium tuberculosis* infections (Vankayalapati et al., 2000; Akgun et al., 2005; Schneider et al., 2010).

In conclusion, we suggest that IL-18 –607 C/A gene promoter polymorphism may not be a major genetic factor for susceptibility to pulmonary tuberculosis in a sample

of Iranian population. Larger studies with different ethnicity are required to validate our findings.

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