ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
February 2015; 14(1):55-59.

Association between Interleukin-1 Receptor Antagonist (IL1RN) Variable Number of Tandem Repeats (VNTR) Polymorphism and Pulmonary Tuberculosis

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Received: 5 December 2013; Received in revised form: 21 January 2014; Accepted: 16 February 2014

ABSTRACT

Macrophages and T-lymphocytes are involved in immune response to Mycobacterium tuberculosis. Macrophage produces interleukin (IL)-1 as an inflammatory mediator. IL-1 receptor antagonist (IL1-Ra) is a natural antagonist of IL-1 receptors. In this study we aimed to examine the possible association between the variable number of tandem repeats (VNTR) of the IL-1 receptor antagonist (IL1RN) gene and pulmonary tuberculosis (TB) in a sample of Iranian population.

Our study is a case-control study and we examined the VNTR of the IL1RN gene in 265 PTB and 250 healthy subjects by PCR.

Neither the overall chi-square comparison of PTB and control subjects nor the logistic regression analysis indicated any association between VNTR IL1RN polymorphism and PTB.

Our data suggest that VNTR IL1RN polymorphism may not be associated with the risk of PTB in a sample of Iranian population. Larger studies with different ethnicities are needed to find out the impact of IL1RN VNTR polymorphism on risk of developing TB.

Keywords: IL1RN; Polymorphism; Tuberculosis; VNTR

INTRODUCTION

Tuberculosis (TB) is one of the most important global public health issues and remains a major cause of death worldwide especially in Asia and Africa. It has been expected that one third of the world's population

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Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

is infected with Mycobacterium tuberculosis (MTB) but approximately 5 to 10% of those infected develop the active disease in their lifetime.[1] TB is an extremely complex disease and it is not well understood why some infected persons develop active disease while others do not. Although pathogens and environmental factors are supposed to contribute to TB, increasing evidence proposes that host genetic factors play a significant role in tuberculosis vulnerability.[2-4] Genetic studies on TB have revealed that the genetic variants, which are potentially involved in the immune response to TB, could be responsible for susceptibility or protection to TB.[4-7]

Interleukins (ILs) are proinflammatory cytokines produced by monocytes, macrophages and epithelial cells. The IL-1 family consists of the cytokines IL-1a, IL-1b and the IL-1 receptor antagonist (IL-1Ra)[8] and the genes encoding this family are mapped on chromosome 2q14.[9] The role of *IL1RN* variable number tandem repeat (VNTR) has being studied in the development of inflammatory disorders for several years.[10-12] The pro-inflammatory cytokine IL-1b and its antagonist, IL1Ra, are strongly induced by M. TB and are encoded by polymorphic genes.[13] A key component in the inflammatory response is the prompt production of pro-inflammatory cytokines such as IL-1b and tumor necrosis factor-α TNF-α, which are required to control infection by M. TB.[14, 15] The pro-inflammatory response is down-regulated by cytokines transforming growth factor-β (TGF-β, IL-10, and, specifically in the case of IL-1, IL1Ra, to terminate the immune response and limit the potential for immunopathology.[16]

The IL1Ra is an important immunologic regulator that competes with other IL-1 family members (IL-1a and IL-1b) for the IL-1 receptor in target cells and acting as its negative regulator with anti-inflammatory effects.[16-18] The IL1RN contains an 86-base pair VNTR polymorphism in intron 2. There is little and controversial data regarding the possible role of IL1RN VNTR polymorphism on TB[7,13,19], thus the present study was designed to find out the possible association between IL1RN VNTR polymorphism and pulmonary TB in a sample of Iranian population.

MATERIALS AND METHODS

This case control study was conducted on 265 patients with pulmonary TB (PTB) and 250 healthy subjects. The local ethics committee of the Zahedan University of Medical Sciences approved the project, and written informed consent was taken from all participants. All control subjects were healthy subjects from the same geographical origin, and were living in the same region as the patients with PTB (Zahedan, southeast Iran). They had no recent signs, symptoms or history of PTB.

The diagnosis of PTB was based on clinical symptoms, radiological evidence, and bacteriological investigations such as sputum Acid Fast Bacillus (AFB) smear positivity, culture and response to antituberculosis chemotherapy. Whole blood samples were collected in Na-EDTA tubes from all subjects and genomic DNA was extracted using salting out method.

The VNTR polymorphisms of IL-1RN were genotyped by polymerase chain reaction (PCR) with 5`-CTCAGCAACACTCCTAT-3` forward primer and 5`-TCCTGGTCTGCAGGTAA-3` reverse primer as previously described.[20]

PCR was done using a PCR premix (AccuPower PCR PreMix, BIONEER, Daejeon, Korea). 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 17 μL DNase-free water were added. PCR was performed in conditions as follows: 95 °C for 5 min; 95 °C for 30 s, 61 °C for 25 s, 72 °C for 30 s, 30 cycles; 72 °C for 10 min. The PCR products were electrophoresed on 2% agarose gels and photograph was taken (Figure 1).

Statistical Analysis

The statistical analysis of the data was performed using the SPSS 18.0 software. Demographic and biochemical parameters between the groups were analyses by independent sample t-test for continuous data and $\chi 2$ test for categorical data. The associations between genotypes and PTB were estimated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. *P*-value less than 0.05 was considered statistically significant.

RESULTS

Two hundred and sixty-five PTB patients (38% males and 62% females; ages 52.4 ± 20.3 years) and 250 healthy subjects (43.7% males and 56.3% females; ages 49.2 ± 16.7 years) were enrolled in the study.

There was no significant difference between cases and controls regarding age and sex (p=0.060 and p=0.268, respectively).

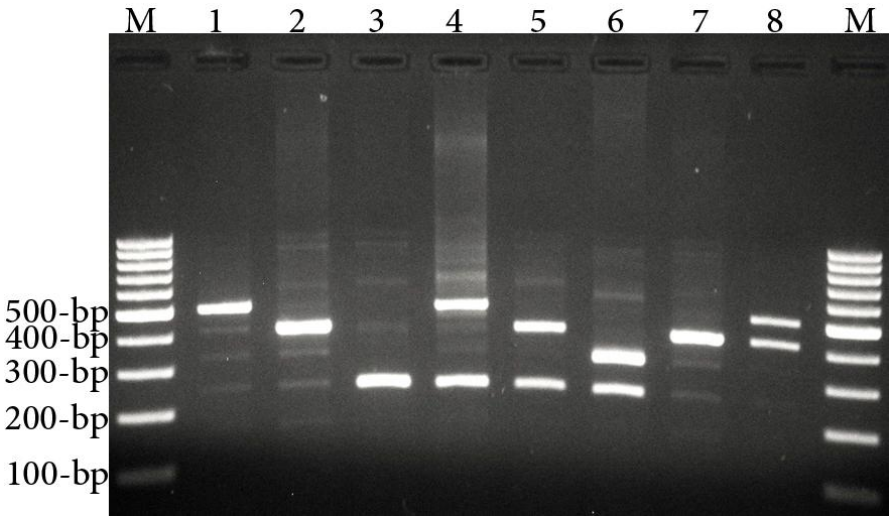

Figure 1. Electrophoresis pattern of PCR product for detection of *IL1RN* VNTR polymorphism
M: DNA Marker; Lane 1: ILRN*3/3; Lanes 2 and 7: ILRN*1/1; Lane 3: ILRN*2/2; Lane 4: ILRN*2/3; Lane 5: ILRN*1/2; Lane 6: ILRN*2/4; Lane 8: ILRN*1/3.

Table 1. Genotype and allele frequencies of the *IL1RN VNTR* polymorphism in PTB and healthy subjects (control)

IL1RN Genotypes	PTB n (%)	Controls n (%)	OR (95% CI)	*P*
IL1RN* 1/1	181 (68.3)	169 (67.6)	1.0	-
IL1RN* 1/2	52 (19.6)	49 (19.6)	0.99 (0.64-1.54)	0.991
IL1RN* 1/3	17 (6.4)	15 (6.0)	1.06 (0.51-2.19)	0.879
IL1RN* 2/2	12 (4.5)	13 (5.2)	1.06 (0.51-2.19)	0.836
IL1RN* 2/3	2 (0.8)	0 (0.0)	-	-
IL1RN* 2/4	0 (0.0)	1 (0.4)	-	-
IL1RN* 3/3	1 (0.4)	3 (1.2)	0.31 (0.03-3.02)	0.314
Alleles				
IL1RN* 1	431 (81.3)	402 (80.4)	1.0	-
IL1RN* 2	78 (14.8)	76 (15.2)	0.96 (0.67-1.35)	0.873
IL1RN* 3	21 (3.9)	21 (4.2)	0.93 (0.50-1.73)	
IL1RN* 4	0 (0.0)	1 (0.2)	-	-

IL1RN, IL-1 receptor antagonist; VNTR, variable number of tandem repeats

According to the differences of 86-bp tandem repeat number, five types of alleles can be recognized. *IL1RN**1 (4 repeats, 420-bp), *IL1RN**2 (2 repeats, 240-bp), *IL1RN**3 (5 repeats, 498-bp), *IL1RN**4 (3 repeats, 326-bp) and *IL1RN**5 (6 repeats, 595-bp).[20]

In the present study, four alleles (*IL1RN**1, *2, *3 and IL1RN*4) were recognized.

The genotype and allele frequencies of IL1RN VNTR polymorphisms in PTB patients and control subjects are shown in table 1. Neither the overall chi-square comparison of PTB and control subjects ($\chi 2=4.32$, $p=0.645$) nor the logistic regression analysis indicated any association between VNTR polymorphisms of *IL1RN* and PTB (Table 1).

DISCUSSION

To the best of our knowledge, there are no reports regarding the impact of *ILRN* VNTR polymorphism and TB in the Iranian population. In the present study, we investigated the impact of IL1RN VNTR polymorphism and PTB in our population. Our findings revealed that there was no significant association between *IL1RN* VNTR polymorphism and PTB. In

agreement to our finding, Awomoyi et al [7] and Wilkinson et al [13] found no association between *ILRN* VNTR variants and TB. Bellamy et al [19] have investigated the impact of *IL1RN* VNTR polymorphism in Gambian population. Their findings showed significantly fewer *IL1RN* allele 2 heterozygotes among the TB cases compared to the controls (comparing the two commonest genotypes 1/1 and 1/2, $\chi 2= 4.71$, $p=0.030$). They concluded that *IL1RN* VNTR polymorphisms marginally correlated with TB.

There is substantial evidence for a role of the intron 2 VNTR in regulation of *IL1RN* expression, though there is debate over the function of each individual allele. Danis et al. [21] reported an association of the *IL1RN**2 allele with increased in vitro release of IL-1Ra from human monocytes. However, Clay et al. [22] found no difference in allele-specific IL1RN mRNA accumulation in keratinocyte cell lines, while Dewberry et al. [23] showed that *IL1RN**2 carriage correlated with decreased mRNA levels of the IL-1Ra isoform in human umbilical vein and aortic endothelial cells. Although the abovementioned findings might appear contradictory, there are at least two plausible explanations for such discrepant findings: namely, that the allelic effect might be cell type-specific and/or that the effect of individual VNTR alleles on IL1RN expression relates to the IL-1Rα isoform being produced. Hurme and Santtila [18] reported higher plasma IL-1Rα levels in healthy carriers of the *IL1RN**2 allele, compared with non- carriers.

Cytokines have various functions as intercellular signaling substances through intracellular signal transduction and the second messenger pathway. IL-1 is a proinflammatory and profibrotic cytokine that exists in two forms: IL-1a and IL-1b. IL1Ra is an inhibitor of IL-1 by competitive binding to the IL-1 receptor. As a consequence, the biologic activity of this cytokine is neutralized in physiologic and pathophysiologic immune and inflammatory responses. The *IL1RN* encoding IL-1Ra protein has been concerned in idiopathic pulmonary fibrosis susceptibility.[24] Variations in IL1RN can modulate the effectiveness of IL-1 signaling and thereby predispose to disease condition. Resistance to M. TB is related to macrophage activation and Th1 response.[25] Cytokines that exert effects upon these cells are therefore good candidates for TB susceptibility. IL1α and IL1b are involved in regulation of immunological and inflammatory reactions by inducing expression of many effector proteins, such as cytokines/chemokines, nitric oxide synthetase and matrix metalloproteinases .[26] They exert their effects by interaction with the IL-1 receptor and are antagonized by IL1Ra.[27] It has been shown that VNTR allele 2 polymorphism of *IL1RN* is associated with a wide range of chronic inflammatory and autoimmune conditions.[20]

In the present study we showed that 86-bp VNTR polymorphism in intron 2 of *IL1RN* was not associated with the risk of PTB in our study population.

In conclusion, we found no significant association between *IL1RN* VNTR polymorphisms and PTB in a sample of Iranian population. Larger studies are needed to validate our findings.

ACKNOWLEDGEMENTS

This work was funded by a research grant from Zahedan University of Medical sciences. The authors thank to the patients and healthy subjects who willingly participated in the study.

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