

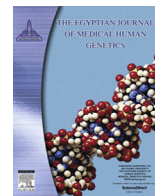
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## The Egyptian Journal of Medical Human Genetics

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Original article

Association of *P2X7* gene common polymorphisms with pulmonary tuberculosis in Lur population of IranAli Amiri<sup>a,b</sup>, Toomaj Sabooteh<sup>c</sup>, Seyyed Amir Yasin Ahmadi<sup>d,e</sup>, Alireza Azargoon<sup>b</sup>, Farhad Shahsavari<sup>f,\*</sup><sup>a</sup> Social Determinants of Health Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran<sup>b</sup> Department of Internal Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran<sup>c</sup> Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran<sup>d</sup> Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran<sup>e</sup> Scientific Society of Evidence-Based Knowledge, Research Office for the History of Persian Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran<sup>f</sup> Department of Immunology, Lorestan University of Medical Sciences, Khorramabad, Iran

## ARTICLE INFO

## Article history:

Received 29 November 2017

Accepted 24 December 2017

Available online 5 January 2018

## Keywords:

Pulmonary tuberculosis

Immunogenetics

*P2X7*

Population genetics

## ABSTRACT

**Background:** Different genetic and environmental factors are associated with susceptibility to pulmonary tuberculosis (TB) in different individuals of different populations. Based on previous studies role of *P2X7* gene common polymorphisms in susceptibility to pulmonary TB was associated with ethnicities.

**Aim:** We intend to perform this study on genetic reservoir (gene pool) of Lur population of western Iran.

**Methods:** For the present case-control study, 100 unrelated pulmonary TB patients and 100 unrelated controls were enrolled through convenient sampling. TB confirmation was through smear and culture of sputum. Polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) was used for molecular assay. This study has been approved in the ethic committee of Lorestan University of Medical Sciences with registration number LUMS.REC.1396.253.

**Results:** Among the genotypes of polymorphism 1513A/C, AA genotype was associated with susceptibility to pulmonary TB ( $P = .0001$ ; OR = 4.750) whereas AC genotype was a protecting factor ( $P = .0001$ ; OR = 0.192). Higher genetic reservoir of A allele was associated with more susceptibility to pulmonary TB ( $P = .0001$ ; OR = 2.879) whereas C allele was a protecting factor ( $P = .0001$ ; OR = 0.347). No significant result was found for -762T/C polymorphism.

**Conclusion:** In Lur population of Iran, 1513A/C polymorphism of *P2X7* is associated with susceptibility to pulmonary TB. It is suggested that bio-information banks should be established and developed in countries.

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## 1. Introduction

## 1.1. Background

Tuberculosis (TB) in general and pulmonary TB in particular, is a reason of mortality worldwide [1]. Based on the report of world health organization (WHO), 9.2 million new cases of TB have been discovered with 1.7 million cases of mortality [2]. It is estimated that one third of world population are infected with mycobacterium TB (as latent TB) that only 5–10% of such infected individuals reach the active and overt disease [3]. The quality and prognosis of its end stage is different among different individuals

[4]. Hence TB is a scientific model of medical anthropology. Different genetic and environmental factors are associated with susceptibility to pulmonary TB in different individuals of different populations (Fig. 1). For instance the involved polymorphic genes are *TNF- $\alpha$* , *TLR*, *VDR*, *MBL*, *NRAMP1*, *CD209* and *IL-10* [5–8].

Purinergic receptor *P2X*, ligand-gated ion channel 7 (*P2X7*) is a receptor belonged to the family of ATP-gated non-selective cation channels that has a high permeability to sodium, potassium and calcium ions [9]. This receptor is highly expressed on surface of blood cells, immune cells and in particular mono-nuclear lymphocytes. Hence it plays its role in pre-inflammatory cytokine release from monocytes and macrophages [10]. *P2X7* is encoded by the *P2X7* which has 13 exons on chromosome 12q24. This gene encodes a 595 aminoacid polypeptide with two trans-membrane domains [11]. Activation of *P2X7* with adenosine triphosphate (ATP) results in rapid opening of cation channels, and hence results in calcium and sodium ions import and potassium ion export. This process

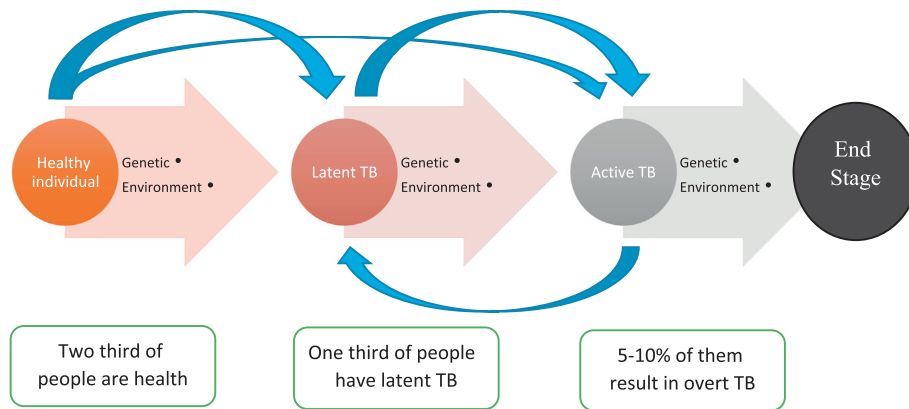
Peer review under responsibility of Ain Shams University.

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**Fig. 1.** Genetic and environmental factors is susceptibility to pulmonary TB at different levels.

is initiator of one of the pathways of caspase cascade resulting in apoptosis; or resulting in the phospholipase D activation in turn resulting in phagosome-lysosome fusion and finally mycobacterium killing [12–15]. *P2X7* gene has two single nucleotide common polymorphisms (SNP) –762C/T and 1513A/C. Polymorphism 1513A/C exists on exon 13 of this gene, and plays a role in susceptibility to pulmonary TB in some populations through affecting *P2X7*, whereas polymorphism –762C/T exists on the upstream [16–20].

## 1.2. Aims

Because of the controversial findings of previous studies which were due to ethnicity effect, we intend to perform this study on genetic reservoir (gene pool) of Lur population of western Iran. It seems that the strength and effect size of this association can be different among different ethnicities.

## 2. Subjects and methods

### 2.1. Subjects

For the present case-control study, 100 unrelated pulmonary TB patients and 100 unrelated controls were enrolled through convenient sampling. The including criteria were being resident in Lorestan province of Iran, and the two recent generations of the participants should be Lur. The exclusion criteria were having any other background disease and not having written consents.

### 2.2. Laboratory analysis

TB confirmation was through smear and culture of sputum. Two ml of peripheral blood of each patient were collected in ethylenediamine tetraacetic acid (EDTA) containing test tubes. DNA samples were extracted and purified using salting-out method. Polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) was used for molecular assay (Table 1). This method has previously been used [21–23].

**Table 1**  
Sequences of the primers used for PCR.

Polymorphism	Sequences of primers	Restriction enzymes	Length (bp)
–762T/C	Outer Primer: F: 5'-GAAACAGGGCCCTGGGTCCTC-3' R: 5'-TGGTGGGGTGGAGGGGC-3'	<i>Hinc II</i>	CC (235) CT (186 + 235) TT (186)
	Inner Primer: F: 5'-GGTGTCCCTCACTGAATAGGTCAAT-3' R: 5'-GGCAGTCCAACAAAGTTAGGTTTG-3'		
1513A/C	F: 5'-ACTCCTAG ATCCAGGGATAGCC-3' R: 5'-TACAGACGTGAGCCACGGT-3'	<i>Hae II</i>	AA (209 + 143+65) AC (209 + 143+117 + 92+65) CC (143 + 117+95 + 65)

### 2.3. Statistical analysis

The frequency of the polymorphisms between groups were compared through chi-square test and reporting the odds ratios (OR) with their 95% confidence intervals (CI). The Iranian studies were compared through dendrogram using STATA14 software (StataCorp LLC, US). This cluster analysis was based on dissimilarity matrix of binary variables through complete linkage method.

### 2.4. Ethical considerations

This study has been approved in the ethics committee of Lorestan University of Medical Sciences with registration number LUMS.REC.1396.253. Based on the comments mentioned in this committee we took informed consent from the participants and observed Helsinki rules.

## 3. Results

### 3.1. Demographic findings

In the present study 100 pulmonary smear positive individuals and 100 healthy controls were evaluated. The mean age of the patients was 32.61 ( $\pm 7.5$ ) years and the mean age of the controls was 28.15 ( $\pm 3.76$ ) years. Male/female ratio were 43/57 and 49/51 in the patients and the controls respectively.

### 3.2. Genetic findings

No significant deviation from Hardy-Weinberg equilibrium was detected ( $P > .05$ ). Among the genotypes of polymorphism 1513A/C, AA genotype was associated with susceptibility to pulmonary TB ( $P = .0001$ ; OR = 4.750) whereas AC genotype was a protecting factor ( $P = .0001$ ; OR = 0.192). From the viewpoint of population genetics, higher genetic reservoir of A allele was associated with more susceptibility to pulmonary TB ( $P = .0001$ ; OR = 2.879).

**Table 2**  
Genotype distribution of P2X7 in patient and control groups.

Polymorphism	Genotype	Frequency in patients	Frequency in controls	Odds ratio	P Value
-762T/C	TT	4	1	4.125	.1742
	TC	88	95	0.386	.0759
	CC	8	4	2.087	.2337
1513A/C	AA	76*	40	4.750	.0001
	AC	21	58*	0.192	.0001
	CC	3	2	1.515	.6506

**Table 3**  
Allele distribution of P2X7 in patient and control groups.

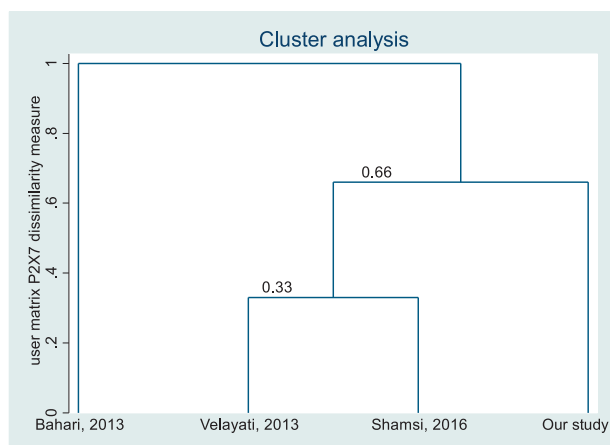
Polymorphism	Allele	Frequency in patients	Frequency in controls	Odds ratio	P value
-762T/C	T	48	48.5	0.980	.9203
	C	52	51.5	1.020	.9203
1513A/C	A	86.5*	69	2.879	.0001
	C	13.5	31*	0.347	.0001

**Table 4**  
Binary variables and dissimilarity matrix of the studies done in Iran about association of P2X7 polymorphisms and susceptibility to pulmonary TB.

Study	Place	Same ethnicity (Fars)	-762C/T associated	1513A/C associated
Bahari, 2013	Zahedan	No (Sistanei)	Yes	No
Velayati, 2013	Tehran	Yes	No	Yes
Shamsi, 2016	Tehran	Yes	Yes	Yes
Our study, 2017	Lorestan	No (Lur)	No	Yes

Dissimilarity matrix				
Study	Bahari, 2013	Velayati, 2013	Shamsi, 2016	Our study, 2017
Bahari, 2013	0	1	0.66	1
Velayati, 2013	1	0	0.33	0.33
Shamsi, 2016	0.66	0.33	0	0.66
Our study, 2017	1	0.33	0.66	0

**Fig. 2.** Cluster analysis of the dissimilarity matrix (Table 4) based on complete linkage method.

whereas C allele was a protecting factor ( $P = .0001$ ;  $OR = 0.347$ ). No significant result was found for -762T/C polymorphism neither for genotypes nor for alleles (Tables 2 and 3). The cluster analysis was done on 4 Iranian studies (Table 4, Fig. 2).

## 4. Discussion

### 4.1. Summary of evidence

This study was aimed to investigate the association of P2X7 polymorphism with susceptibility to pulmonary TB in Lur

population of Iran. It had been hypothesized that the strength and effect size of this association could be different among ethnicities. West of Iran has different populations of Kurd, Lur and Lak. Previously we had been reported the human leukocyte antigen (HLA) neighbor-joining tree of these ethnicities [24,25]. Based on this, such people were closely like to total Iranian population but still with unique diversities.

Li et al. (2002) conducted a study on 300 Gambian patients of pulmonary TB. They concluded that C allele and CC genotype of -762C/T polymorphism was associated with TB [26]. In contrast to them, we found no significant relation for this polymorphism. In our population most people were heterozygote for this polymorphism. Xiao et al. (2009) investigated this association on 96 patients of Han population of China. They found no significant correlation neither for -762C/T nor 1513A/C [27]. Sambasivan et al. (2010) performed this study on 156 Indian patients. They reported that C allele of -762C/T polymorphism was associated with susceptibility to pulmonary TB; but they found no significant relation for polymorphism 1513A/C [28]. As well, Wang et al. (2011) found no significant relation for these polymorphisms [12]. In Iran, Bahari et al. (2013) on 150 patients of Zahedan, east of Iran, reported a significant relation for polymorphism -762C/T, but not for 1513A/C [29]. It seems that in Lur population of Iran, 1513A/C polymorphism can be more effective even in contrast to this population of Iran. The cluster analysis shows that Bahari et al. study has different results in comparison to other Iranian populations [21,30]. This may be due different ethnicities or maybe technical bias.

### 4.2. Strength and limitations

The strength and novelty of our study was that we could show a different genetic reservoir in Lur population of Iran and its

association with susceptibility to or protection against pulmonary TB. From the limitations of ours, we can point out to this fact that we did not have any subgroup of pulmonary TB mentioned in Fig. 1.

## 5. Conclusion

In Lur population of Iran, 1513A/C polymorphism of P2X7 is associated with susceptibility to pulmonary TB, which is unique in comparison to some other populations. Such findings can help health managers of countries to decide based on their genetic reservoirs. It is suggested that bio-information banks should be established and developed in countries.

## Acknowledgments

We are grateful to all of the patients and individuals for their participation. As well we appreciate Dr. Razieh Shabani. This study was supported by the Lorestan University of Medical Sciences under Grant No. 1647-4.

## Conflict of interest

None.

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