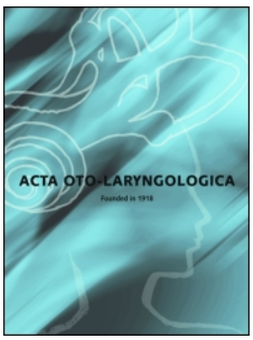

Acta Oto-Laryngologica

ISSN: 0001-6489 (Print) 1651-2251 (Online) Journal homepage: https://www.tandfonline.com/loi/ioto20

MBL, P2X7, and SLC11A1 gene polymorphisms in patients with oropharyngeal tularemia

Battal Tahsin Somuk, Sema Koc, Omer Ates, Göksel Göktas, Harun Soyalic, Ismail Onder Uysal, Levent Gurbuzler, Emrah Sapmaz, Saime Sezer & Ahmet Eyibilen

To cite this article: Battal Tahsin Somuk, Sema Koc, Omer Ates, Göksel Göktas, Harun Soyalic, Ismail Onder Uysal, Levent Gurbuzler, Emrah Sapmaz, Saime Sezer & Ahmet Eyibilen (2016) MBL, P2X7, and SLC11A1 gene polymorphisms in patients with oropharyngeal tularemia, Acta Oto-Laryngologica, 136:11, 1168-1172, DOI: 10.1080/00016489.2016.1186835

To link to this article: https://doi.org/10.1080/00016489.2016.1186835

Published online: 25 May 2016.

ACTA OTO-LARYNGOLOGICA, 2016
VOL. 136, NO. 11, 1168–1172
http://dx.doi.org/10.1080/00016489.2016.1186835

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

MBL, P2X7, and SLC11A1 gene polymorphisms in patients with oropharyngeal tularemia

Battal Tahsin Somuk[a], Sema Koc[b], Omer Ates[c], Göksel Göktas[a], Harun Soyalic[d], Ismail Onder Uysal[e], Levent Gurbuzler[a], Emrah Sapmaz[a], Saime Sezer[c] and Ahmet Eyibilen[a]

[a]Faculty of Medicine, Department of Otorhinolaryngology, Gaziosmanpasa University, Tokat, Turkey; [b]Department of Otorhinolaryngology, Antalya Training and Research Hospital, Antalya, Turkey; [c]Faculty of Medicine, Medical Biology and Genetics, Gaziosmanpasa University, Tokat, Turkey; [d]Faculty of Medicine, Department of Otorhinolaryngology, Ahi-Evran University, Kirsehir, Turkey; [e]Faculty of Medicine, Department of Otorhinolaryngology, Cumhuriyet University, Sivas, Turkey

ABSTRACT

Conclusion: A significant association was found of oropharyngeal tularemia with SLC11A1 allele polymorphism (INT4 G/C) and MBL2 C + 4T (P/Q). These results indicate C allele and Q allele might be a risk factor for the development of oropharyngeal tularemia.

Aim: This study aimed to investigate the relationship of SLC11A1, MBL, and $P2X_7$ gene polymorphism with oropharyngeal tularemia.

Methods: The study included totally 120 patients who were diagnosed with oropharyngeal tularemia. Frequencies of polymorphisms in the following genes were analyzed both in the patient and control groups in the study: SLC11A1 (5′$(GT)_n$ Allele 2/3, Int4 G/C, 3′ UTR, D543N G/A), MBL (MBL2 C + 4T (P/Q), and $P2X_7$ (−762 C/T and 1513 A/C).

Results: Among all polymorphisms that were investigated in this study, SLC11A1 gene showed a significance in the distriburtion of polymorphism allelle frequency at the INT4 region. Frequency of C allele was 54 (28%) in patients with oropharyngeal tularemia, and 31 (13%) in the control group ($p = 0.006$ and OR = 1.96 (1.21–3.20)). An association was detected between MBL2 C + 4T (P/Q) gene polymorphism and oropharyngeal tularemia ($p < 0.005$ and OR = 0.30 (0.19–0.48)). No significant relation was found between $P2X_7$ (−762 C/T and 1513 A/C) gene polymorphism and oropharyngeal tularemia in this study ($p > 0.05$).

ARTICLE HISTORY
Received 4 April 2016
Revised 25 April 2016
Accepted 27 April 2016
Published online 23 May 2016

KEYWORDS
MBL genotypes; NRAMP1 (SLC11A1); $P2X_7$; polymorphism; tularemia

Introduction

Tularemia is a zoonotic disease that is caused by the bacteria *Francisella tularensis*, which is an immotile, aerobic, and Gram-negative intracellular coccobacillus. The macrophage cytosol is a suitable place for the reproduction of this bacteria because it provides the necessary ions for its reproduction while protecting it from the immune system's attacks [1]. The bacteria is also an intracellular pathogen. The main route for tularemia's development is through reproduction of the bacteria within mononuclear phagocytes, such as macrophages and dendritic cells. Nevertheless, the bacteria can also infect neutrophils, hepatocytes, and epithelial and endothelial cells. Delay in its diagnosis, in addition to inadequate treatment, can lead to high rates of morbidity and mortality [2].

The tularemia agent is extremely virulent and can be transmitted to humans in various ways. The most common routes of its transmission are through either contact with infected ticks and animals or through consumption of contaminated food and water [3]. Tularemia has six clinical forms. These include glandular, typhoidal, pneumonic, oropharyngeal, and oculoglandular tularemia. The form that is most commonly encountered by ear, nose, and throat physicians is oropharyngeal tularemia. The most significant complaints observed in this clinical form include fevers and sore throats. During physical examinations, membranous tonsillitis and generally unilateral cervical lymphadenopathy can be observed [4]. Following inoculation to tularemia, the inflammatory cells are the first to arrive at the site of infection. Neutrophils and macrophages then surround the early inflammatory cells that are undergoing necrosis and degeneration. Later, giant, epithelioid, and lymphocyte cells migrate to the necrotic tissue and granulomatous infections progress.

Due to its histopathological features, Tularemia can be confused with tuberculosis. Cervical lymphadenopathy that develops secondarily to oropharyngeal tularemia shares several clinical and histopathological similarities with tuberculous lymphadenitis [5]. Both diseases are caused by intracellular bacteria, and both are linked to chronic granulomatous infections. $P2X_7$, mannose-binding lectin (MBL), and solute carrier family 11 member 1 (SLC11A1) genes are vital to the pathogenesis of intracellular infections. Several studies that have evaluated different populations have shown an association between these genes and tuberculosis [6–9]. These studies suggest that each gene polymorphism is likely responsible for oropharyngeal tularemia, which has similar clinical and histopathological features with tuberculosis.

CONTACT Battal Tahsin Somuk, MD btahsin@hotmail.com Faculty of Medicine, Department of Otorhinolaryngology, Gaziosmanpasa University, Muhittin Fisunoğlu Street, Ali Şevki Erek's Campus, Tokat, Turkey

Table 1. Primer sequences and restriction enzymes for NRAMP-1 5′(GT)n Allel2/3, Int4 G/C, 3′ UTR and D543N G/A; P2X7 −762 C/T and 1513 A/C; MBL2 C+4T (P/Q) polymorphisms.

Polymorphisms	Primers	PCR product (bp)
3′UTRS1	5′-TGA CTG GCC TGC TGG ATG T-3′	103 bp
3′UTRG	5′- GAA ACA GCA GGT CCC TAA AG-3′	
3′UTRS2	5′- GAC TGG CCT GCT GGA GAG-3′	
D543NS1	5′- TGT ATG GGC TCC TTG AAG AGG-3′	190 bp
D543NG	5′-GAA ACA GCA GGT CCC TAA AG-3′	
D543NS2	5′-GTA TGG GCT CCT TGA AGA GA-3′	
INT4S1	5′-GGT TCT CCC TGT CCA GGC-3′	166 bp
INT4G	5′-CCT GCC TCC TCA CAG CTT CT-3′	
INT4S2	5′-GGT TCT CCC TGT CCA GGG-3′	
IKP1	5′-GCC TTC CCA ACC ATT CCC TTA-3′	Human Growth Hormone Gene (ARMS-PCR control) 429 bp
IKP2	5′-TCA CGG ATT TCT GTT GTG TTC C-3′	
P2X74	5-ATGTGCGTAGCTCTTCTGGTG-3	126 bp (HincII) 126/80/46
−762	5-GGCAGGTCGATCTATGACCTA-3	
P2X74	5-AGACCTACGATGGACTTCACAG-3	316 bp (HaeII) 316/200/119
1513A/C	5-AGCGCCAGCAAGGGCTC-3	
MBL2 C + 4T (P/Q)	P 5-CAGATTGTAGGACAGAGGGCATGCTC-3	P 335 bp
	Q 5-TTGTAGGACAGAGGGCATGCTT-3	Q 331 bp
	G 5-CCAGGCAGTTTCCTCTGGAAGG-3	

Gene polymorphisms are single base-pair alterations within genomic DNA and can differ among normal individuals within a given population. There are many studies that indicate the associations of MBL, $P2X_7$, and SLC11A1 gene polymorphisms with infectious diseases caused by tubercle bacillus, which is an intracellular pathogen. The present study examined the frequency of MBL (MBL2 C + 4T (P/Q), $P2X_7$ (−762 C/T and 513 A/C), and SLC11A1 ($5'(GT)_n$ Allele2/3, Int4 G/C, 3′ UTR, D543N G/A) gene polymorphisms among patients with oropharyngeal tularemia and investigated the association of these gene polymorphisms with oropharyngeal tularemia.

Materials and methods

Patient and controls

The study included a total of 120 patients who had been diagnosed with oropharyngeal tularemia between January 2010 and January 2013 (mean age = 34.66 ± 18.99 years; age range = 2–84; 64 males (53.3%); 56 females (46.7%)), and 120 healthy volunteers as the control group (mean age = 36.33 ± 19.67 years; age range = 2–86; 68 males (56.7%); 52 females (43.3%)). For patients whose symptoms were suggestive of tularemia (fever, lymphadenitis, acute tonsillopharyngitis), definitive diagnoses were made upon Polymerase chain reaction (PCR) and serological examinations of blood and lymph node aspiration samples at the national reference laboratory. For differential diagnoses of tuberculous lymphadenitis, blood, tissue, and lymph node aspiration samples were sent for PCR analyses and histopathological examinations. Individuals in both patient and control groups were excluded from the study if they had chronic inflammatory, autoimmune, and infectious diseases or diabetes mellitus, hypertension, and cardiac diseases. This study was approved by the instititutional review board and ethics committee of our institution, and informed consent was obtained from all patients. Every individual provided a 5 ml blood sample that was collected into a tube containing ethylenediaminetetraacetic acid (EDTA). The study plan was approved by the local ethics committee (approval number: 13-KAEK-239) and informed consent was obtained from all of the women that participated in the study.

Genetic analysis

Genomic DNA was isolated from peripheral blood using Fermentas DNA isolation kit. We used the ARMS-PCR method for analyzing the polymorphisms of NRAMP-1 Int4 G/C, 3′ UTR and D543N G/A, MBL2 C + 4T (P/Q), and RFLP-PCR method for analyzing the polymorphism of $P2X_7$ −762 C/T and 1513 A/C. The PCR was performed with a 25-μL reaction mixture containing 50 ng of genomic DNA, 0.8 nmol/μl of each primer, 10X reaction Buffer, 1.5 mM $MgCI_2$, 0.3 mM dNTP, and 1U Taq DNA polymerase (Fermentase). Primer sequences and restriction enzymes for NRAMP-1 $5'(GT)_n$ Allel2/3, Int4 G/C, 3′ UTR and D543N G/A; $P2X_7$ −762 C/T and 1513 A/C; MBL2 C + 4T (P/Q) polymorphisms were shown in Table 1. PCR programs for NRAMP-1 $5'(GT)_n$ Allel2/3, Int4 G/C, 3′ UTR and D543N G/A; $P2X_7$ −762 C/T and 1513 A/C; MBL2 C + 4T (P/Q) polymorphisms were shown in Table 2.

Statictical analysis

Statistical analysis was performed by Epi Info Software Version 20.0 (CDC, Atlanta, GA). The distribution of NRAMP-1 $5'(GT)_n$ Allel2/3, Int4 G/C, 3′ UTR and D543N G/A; $P2X_7$ −762 C/T, and 1513 A/C; MBL2 C + 4T (P/Q) polymorphisms between patients with tularemia and healthy controls were compared by the χ^2 or Fischer's exact test. The genotype distribution and Hardy–Weinberg equilibrium were tested with the χ^2 test for quality of fit, Arlequin Software v. 2000 (University of Geneve, Geneva, Switzerland).

Results

We examined the MBL (MBL2 C + 4T (P/Q), $P2X_7$ (−762 C/T and 1513 A/C), and SLC11A1 ($5'(GT)_n$ Allele2/3,

Table 2. PCR programmes for NRAMP-1 5′(GT)n Allel2/3, Int4 G/C, 3′ UTR and D543N G/A; P2X7–762 C/T and 1513 A/C; MBL2 C+4T (P/Q) polymorphisms.

PCR program	NRAMP-1 5′$(GT)_n$ Allel2/3	D543N, 3′UTR ve INT4	P2X7 −762 C/T	1513 A/C	MBL2 C + 4T (P/Q)
İnitial denaturation	94 °C 2 min	94 °C 2 min	94 °C 3 min	94 °C 3 min	95 °C 10 min
Denaturation	94 °C 1 min	94 °C 10 s	94 °C 30 s	94 °C 30 s	94 °C 30 s
Annealing	58 °C 60 s	65 °C 1 min	59 °C 45 s	56 °C 30 s	66 °C 30 s
Elongation	72 °C 60 s	94 °C 10 s 61 °C 50 s 72 °C 30 s	72 °C 45 s	72 °C 45 s	72 °C 45 s
Final elongation	72 °C 10 min	72 °C 5 min	72 °C 5 min	72 °C 5 min	72 °C 5 min
Cycles	28 cycles	10 cycles 20 cycles	35 cycles	35 cycles	30 cycles

Table 3. Genotype and allele frequencies of NRAMP-1 5′(GT)n Allel2/3, Int4 G/C, 3′ UTR and D543N G/A; P2X7–762 C/T and 1513 A/C; MBL2 C+4T (P/Q) gene polymorphisms in tularemia patients and control groups.

	Tularemia patients ($n = 120$)	Control group ($n = 120$)	p	OR (95% CI)
NRAMP-1 5′$(GT)_n$ Allel2/3			0.3395	
Allele 2/2	5 [4%]	4 [3%]		
Allele 2/3	48 [40%]	38 [32%]		
Allele 3/3	67 [56%]	78 [65%]		
Allele frequencies			0.093	
Allele 2	58 [24%]	46 [19%]		
Allele 3	182 [76%]	194 [81%]		
NRAMP-1 Int4 G/C			0.014	
GG	71 [59%]	90 [75%]		
GC	44 [37%]	29 [24%]		
CC	5 [4%]	1 [1%]		
Allele frequencies			0.003	0.51 (0.31–0.83)
G	186 [78%]	209 [87%]		
C	54 [22%]	31 [13%]		
NRAMP-1 3′ UTR			0.375	
D/D	0 [0%]	0 [0%]		
ND/D	5 [4%]	4 [3%]		
ND/ND	115 [96%]	116 [97%]		
Allele frequencies			0.376	
D	5 [2%]	4 [2%]		
ND	235 [98%]	236 [98%]		
NRAMP-1 D543N G/A			0.375	
AA	0 [0%]	0 [0%]		
AG	5 [4%]	4 [3%]		
GG	115 [96%]	116 [97%]		
Allele frequencies			0.376	
A	5 [2%]	4 [2%]		
G	235 [98%]	236 [98%]		
P2X7 −762 C/T			0.2026	
CC	69 [57%]	59 [49%]		
CT	31 [26%]	44 [37%]		
TT	20 [17%]	17 [14%]		
Allele frequencies			0.246	
C	169 [70%]	162 [68%]		
T	71 [30%]	78 [32%]		
P2X7 1513 A/C			0.2353	
AA	68 [57%]	80 [67%]		
AC	44 [37%]	36 [30%]		
CC	8 [6%]	4 [3%]		
Allele frequencies			0.039	0.67 (0.43–1.04)
A	180 [75%]	196 [82%]		
C	60 [25%]	44 [18%]		
MBL2 C + 4T (P/Q)			0.0000	
MBL2 + 4 P/P	49 [41%]	90 [75%]		
MBL2 + 4 P/Q	63 [53%]	29 [24%]		
MBL2 + 4 Q/Q	8 [6%]	1 [1%]		
Allele frequencies			0.0000	0.30 (0.19–0.48)
P	161 [67%]	209 [87%]		
Q	79 [33%]	31 [13%]		

D: deletion; ND: non-deletion; CI: confidence interval; OR: odds ratio.

Int4 G/C, 3′ UTR, D543N G/A) gene polymorphisms in 120 healthy controls and in 120 patients with oropharyngeal tularemia. The NRAMP-1 5′$(GT)_n$ Allel2/3, Int4 G/C, 3′ UTR and D543N G/A; $P2X_7$ −762 C/T and 1513 A/C; MBL2 C + 4T (P/Q) genotypic and allelic frequencies in patients with tularemia and controls were shown in Table 3.

Discussion

While oropharyngeal tularemia can be contracted through the consumption of contaminated meat and water, waterborne epidemics have been reported in a variety of geographical locations [10]. The most common clinical signs of oropharyngeal tularemia are fever and cervical lymphadenopathy [5]. Therefore, tularemia should be considered by ear, nose, and throat practices during the differential diagnoses of several diseases that cause cervical lymphadenopathy (lymphoma, tuberculosis, malignancies, toxoplasmosis, cat scratch disease). Early diagnoses and treatments can prevent morbidity. In cases where lymph node abscesses fail to regress in spite of treatment, surgical drainage may prove helpful [11].

Natural resistance associated macrophage protein 1 (NRAMP1) genes are integral membrane proteins that are localized in the endolysosomal compartments of macrophages. Since its protein is a metal ion carrier, the NRAMP1 gene is also known as Solute Carrier Family 11 Member 1 (SLC11A1) [6]. NRAMP1 is important due to its macrophage-mediated natural immunity against various intracellular pathogens [12]. It is thought to function as a metal ion carrier that mediates iron and magnesium intake in the presence of protons (H+). It also produces iron out of phagolysosomes and pumps it into the plasma of infected macrophages, therefore reducing the iron that is necessary for bacterial reproduction in phagolysosomes. NRAMP1 is also responsible for pumping metals, such as zinc and copper, out of phagolysosomes. These metals are a necessary part of the antioxidant defense mechanisms (Superoxide dismutase, catalase) that the bacteria uses to protect itself from active oxygen compounds, from phagosomes into plasma [13]. Additionally, NRAMP1 plays several significant roles in the macrophage activation pathway, regulating CXC chemokine (α-chemokine) and mediating the release of Interleukin-1beta, MHCII molecules, TNF-α and nitric oxide (NO), and inducible nitric oxide synthase (iNOS) [14].

Due to the aforementioned roles of the NRAMP1 (SLC11A1) gene, it has been thought to play an important role in infectious diseases [15]. In regards to the 3′ UTR and 5′$(GT)_n$ Allel2/3, D543N G/A regions of the SLC11A1 gene, the current study found no significant differences between the control group and the patients with oropharyngeal

tularemia. However, the frequency of C allele in the Int4 G/C region of the gene was significantly high. This caused us to consider that the presence of C allele in the Int4 G/C gene region made the occurrence of oropharyngeal tularemia likely. Tuberculosis has similar clinical and histological properties with tularemia, and studies involving patients with tuberculosis have found many associations between the disease and the Int4 [6]. However, Ateş et al. [6] reported that the links between tuberculosis and the 3′ UTR and D543N regions were not significant within the Turkish population. In a meta-analysis that evaluated the relationship between tuberculosis and SLC11A1 gene polymorphism, Meilang et al. [16] reviewed a total of 82 case control studies and discovered relationships between tuberculosis and four of the SCLC11A1 gene regions.

$P2X_7$ is a protein with a trimetric structure. It is expressed on the cellular membrane, is known to be related to cellular immunity, and is generally activated with adenosine triphosphate (ATP). $P2X_7$ plays a vital role in the apoptosis of macrophages that are infected with intracellular micro-organisms [17]. The current study could not detect a significant association between oropharyngeal tularemia and the $P2X_7$ (−762 C/T ve 1513 A/C) gene polymorphism. In a study that involved Turkish patients with tuberculosis, Özdemir et al. [18] found no links between tuberculosis and the 1513 A/C polymorphism of the $P2X_7$ gene. On the other hand, while Nino-Moreno et al. [9] established no connections between tuberculosis and $P2X_7$ −762 C/T, they did determine a significant relationship between tuberculosis and the 1513 A/C polymorphism.

MBL is an acute phase protein that is synthesized within the liver. It has a molecular weight of 96 kDa and is accepted to be an important component of the natural immune system. MBL is related to the complement system and can act as an opsonin in order to facilitate the phagocytosis of micro-organisms through macrophages [8]. In humans, gene coding members of this family are clustered in the long arm of chromosome 10. Some studies have shown that the heterozygosity of variant MBL alleles can have protective effects against tuberculosis [19]. The current study discovered a significant link between oropharyngeal tularemia and the MBL2 C + 4T (P/Q) gene polymorphisms. When compared to the control group, while the Q allele was significantly more frequent among the patient group, the frequency of P allele was significantly lower in this group.

Søborg et al.'s [20] study confirmed a significant association between MBL2 and tuberculosis. In contrast, Cosar et al. [7] reported that low levels of MBL decreased risks of contracting tuberculosis, particularly in pediatric patient groups from Turkey with extrapulmonary tuberculosis. MBL is vital to the process of macrophage phagocytosis. Another study reported that levels of inflammatory cytokines, such as TNF-α, interleukin-6, and interleukin-10, changed with increasing levels of MBL. It stated that the AA genotype associated with high levels of MBL played an immune-mediating role for patients with tuberculosis [8].

In conclusion, not forgetting the low number of patients studied, our study reflects that SLC11A1 (INT4 G/C) and MBL2 C + 4T (P/Q) polymorphisms may be one of the many genetic factors for tularemia susceptibility in a Turkish population. As several genetic factors are involved in tularemia, the present study is the first that reports the association of SLC11A1 (INT4 G/C) and MBL2 C + 4T (P/Q) polymorphisms with tularemia and our results need to be verified with further studies having larger sample sizes. Because genetic factors that shape the likelihood of tularemia may show variances across different populations, determination of these factors in different populations could expedite advancements.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding information

This study was supported by the Gaziosmanpasa University (project no: 2014/14).

References

[1] Chong A, Celli J. The francisella intracellular life cycle: toward molecular mechanisms of intracellular survival and proliferation. Front Microbiol 2010;1:138.

[2] Sahin M, Atabay HI, Bicakci Z, Unver A, Otlu S. Outbreaks of tularemia in Turkey. Kobe J Med Sci 2007;53:37–42.

[3] Nigrovic LE, Wingerter SL. Tularemia. Infect Dis Clin North Am 2008;22:489–504.

[4] Leblebicioglu H, Esen S, Turan D, Tanyeri Y, Karadenizli A, Ziyagil F, et al. Outbreak of tularemia: a case-control study and environmental investigation in Turkey. Int J Infect Dis 2008;12:265–9.

[5] Koc S, Duygu F, Sogut E, Gurbuzler L, Eyibilen A, Aladag I. Clinical and laboratory findings of tularemia: a retrospective analysis. Kulak Burun Bogaz Ihtis Derg 2012;22:26–31.

[6] Ates O, Dalyan L, Musellim B, Hatemi G, Turker H, Ongen G, et al. NRAMP1 (SLC11A1) gene polymorphisms that correlate with autoimmune versus infectious disease susceptibility in tuberculosis and rheumatoid arthritis. Int J Immunogenet 2009;36:15–19.

[7] Cosar H, Ozkinay F, Onay H, Bayram N, Bakiler AR, Anil M, et al. Low levels of mannose-binding lectin confers protection against tuberculosis in Turkish children. Eur J Clin Microbiol Infect Dis 2008;27:1165–9.

[8] Selvaraj P, Jawahar MS, Rajeswari DN, Alagarasu K, Vidyarani M, Narayanan PR. Role of mannose binding lectin gene variants on its protein levels and macrophage phagocytosis with live Mycobacterium tuberculosis in pulmonary tuberculosis. FEMS Immunol Medical Microbiol 2006;46:433–7.

[9] Nino-Moreno P, Portales-Perez D, Hernandez-Castro B, Portales-Cervantes L, Flores-Meraz V, Baranda L, et al. P2X7 and NRAMP1/SLC11 A1 gene polymorphisms in Mexican mestizo patients with pulmonary tuberculosis. Clin Exp Immunol 2007;148:469–77.

[10] Tarnvik A, Chu MC. New approaches to diagnosis and therapy of tularemia. Ann N Y Acad Sci 2007;1105:378–404.

[11] Helvaci S, Gedikoglu S, Akalin H, Oral HB. Tularemia in Bursa, Turkey: 205 cases in ten years. Eur J Epidemiol 2000;16:271–6.

[12] Blackwell JM, Barton CH, White JK, Searle S, Baker AM, Williams H, et al. Genomic organization and sequence of the human NRAMP gene: identification and mapping of a promoter region polymorphism. Mol Med 1995;1:194–205.

[13] Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, et al. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 1997;388:482–8.

[14] Blackwell JM, Searle S. Genetic regulation of macrophage activation: understanding the function of Nramp1 (=Ity/Lsh/Bcg). Immunol Lett 1999;65:73–80.

[15] Hatta M, Ratnawati Tanaka M, Ito J, Shirakawa T, Kawabata M. NRAMP1/SLC11A1 gene polymorphisms and host susceptibility to *Mycobacterium tuberculosis* and *M. leprae* in South Sulawesi, Indonesia. Southeast Asian J Trop Med Public Health 2010;41:386–94.

[16] Meilang Q, Zhang Y, Zhang J, Zhao Y, Tian C, Huang J, et al. Polymorphisms in the SLC11A1 gene and tuberculosis risk: a meta-analysis update. Int J Tuberc Lung Dis 2012;16:437–46.

[17] Buell GN, Talabot F, Gos A, Lorenz J, Lai E, Morris MA, et al. Gene structure and chromosomal localization of the human P2X7 receptor. Recept Channels 1998;5:347–54.

[18] Ozdemir FA, Erol D, Konar V, Yuce H, Kara Senli E, Bulut F, et al. Lack of association of 1513 A/C polymorphism in P2X7 gene with susceptibility to pulmonary and extrapulmonary tuberculosis. Tuberk Toraks 2014;62:7–11.

[19] Garred P, Richter C, Andersen AB, Madsen HO, Mtoni I, Svejgaard A, et al. Mannan-binding lectin in the sub-Saharan HIV and tuberculosis epidemics. Scand J Immunol 1997;46:204–8.

[20] Soborg C, Andersen AB, Range N, Malenganisho W, Friis H, Magnussen P, et al. Influence of candidate susceptibility genes on tuberculosis in a high endemic region. Mol Immunol 2007;44:2213–20.