Association Study of MBL2 Gene Polymorphisms and Risk of Tuberculosis in Southeast of Iran

Mohsen Taheri[1,2], Rezvan Karimloo[3], Hosna Sarani[4], Behrouz Molashahi[1], Mohammad Naderi[3], Gholamreza Bahari[4], Mohammad Hashemi[1†]

[1]Genetics of Non-Communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran;
[2]Department of Genetic, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran;
[3]Infectious Diseases and Tropical Medicine Research Center, Resistant Tuberculosis Institute, Zahedan University of Medical Sciences, Zahedan, Iran;
[4]Children and Adolescent Health Research Center, Resistant Tuberculosis Institute, Zahedan University of Medical Sciences, Zahedan, Iran
[†]Deceased

Abstract: Mannose-binding lectin (MBL) is an acute phase protein which recognizes the pathogens through its carbohydrate recognition domain. It is an important part of human innate immunity. The aim of the current study was to evaluate the impact of MBL2 polymorphism on pulmonary tuberculosis in a number of patients from the southeast of Iran. In this case-control study, 2 MBL gene polymorphisms (rs1800450, rs7095891) were genotyped using PCR-RFLP method and polymerase chain reaction for detection of 34bp ins/del of MBL2 gene (rs777980157) polymorphism. The study included 170 patients with PTB (pulmonary tuberculosis) and 175 control subjects. The findings indicated that the GA (GA vs. GG: OR=0.172, 95% CI=0.107–0.275, P<0.001) (OR – odds ratio; CI – confidence interval) genotype as well as GA+AA (GA+AA vs. GG: OR=0.191, 95% CI=0.120–0.302, P<0.001)

genotype of rs1800450 reduced the risk of PTB compared to GG genotype. The rs7095891 variant significantly decreased the risk of PTB in codominant (GA vs. GG: OR=0.118, 95% CI=0.054–0.258, P<0.001; and AA vs. GG: OR=0.029, 95% CI=0.01–0.082, P<0.001), dominant (GA+AA vs. GG: OR=0.095, 95% CI=0.044–0.207, P<0.001) and recessive (AA vs. GA+GG: OR=0.172, CI=0.081–0.365, P<0.001) inheritance models. No significant relationship was identified between the rs777980157 variant and PTB risk/protection. In conclusion, we found that the MBL2 rs1800450 and rs7095891 polymorphisms provide relative protection against PTB. Additional studies on larger populations with different ethnicities are required to verify our findings.

Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) infection, which is a major public health trouble in many countries. Base on World Health Organization (WHO) Global Tuberculosis Report, tuberculosis with 7 million new cases and 1.2 million deaths annually is the second-leading cause of death around the world (Huang et al., 2016; World Health Organization, 2019). However, one third of the world's population is infected with Mtb, where most of them do not develop active tuberculosis and while 5–15% of infected individuals develop the clinical disease (Zhou et al., 2018). In Zahedan (the capital of Sistan and Baluchistan province), the incidence rate of TB is higher than in other regions of Iran and tuberculosis infection is one of the major public problems. Environment and host genetics factors may contribute to the high prevalence of *Mycobacterium tuberculosis* in this region (Metanat et al., 2012; Hashemi et al., 2015; Kouhpayeh et al., 2016). Innate immunity is the first line of defense against infection with Mtb. Mannose-binding lectin 2 (MBL2) belongs to a family of proteins termed collectins in the C-type lectin superfamily and produced in the liver (Liu et al., 2016). MBL2 is an acute-phase protein, an important part of the innate immunity and has a key role in host defense against pathogens (Zheng et al., 2018). After entrance of microorganism to body, MBL2 recognizes the bacterial mannose residues by its carbohydrate recognition domain (Guo et al., 2017) and activate the complement cascade through the lectin pathway while also promoting opsonization and phagocytosis of pathogens (Li et al., 2018). The MBL2 gene is located on chromosome 10 (10q11.2-q21) and contains many polymorphisms in the promoter or structural region of the gene affecting the expression level of MBL2 at transcription level. Low serum level of MBL2 is associated with increased susceptibility to infectious disease such as Mtb (Amiri et al., 2017). Several studies have shown that MBL2 polymorphisms are related to tuberculosis susceptibility though the results were inconsistent (Cao et al., 2018; Mandal et al., 2019; Tong et al., 2019). Thus, we conducted the present study to investigate the possible association between MBL2 gene polymorphisms and pulmonary tuberculosis in a sample of Iranian population.

Material and Methods

A total of 170 patients diagnosed with PTB (pulmonary tuberculosis) were enrolled in the present study from May 2017 to December 2018 who referred to university-affiliated hospital center for TB (Bou-Ali Hospital, Zahedan, Iran). Additionally, during the same time period as the TB patients were collected, 175 healthy subjects with no history of TB or pulmonary disease were recruited as control subjects. Controls were selected from the equal region as the patients with PTB (Southeast Iran); they were not related to each other (family members) and they had the same conditions including socioeconomic status and availability of health accommodations. All participants received BCG vaccination.

Pulmonary tuberculosis was confirmed by clinical appearances, chest X-ray sign, and positive sputum smear for acid-fast bacilli as described in our previous study (Kouhpayeh et al., 2012). Informed consent was taken from all subjects and the project was approved by the local Ethics Committee of Zahedan University of Medical Sciences (IR.ZAUMS.REC.1396.216). About 2 ml peripheral blood was taken from each patient and control, DNA extracted using salting out methods. In this study, we used polymerase chain reaction for detection of 34bp ins/del of MBL2 gene (rs777980157) polymorphism. Genotyping of rs1800450 A/G and rs7095891 A/G MBL2 gene polymorphisms was done by PCR-RFLP method. Primer sequences, restriction enzymes, and length of the fragments are summarized in Table 1. In each 0.20-ml PCR reaction tube, 1 μl of genomic DNA (~100 ng/ml), 1 μl of each primer (10 μM), and 10 μl of 2X Prime Taq Premix (Genet Bio, Korea), and 7 μl ddH$_2$O were added. The thermal cycling parameters consisted of an initial denaturation at 95 °C for 5 min, 30 cycles of 95 °C for 30 s, 56 °C for rs777980157, 64 °C for rs1800450 and 61 °C for rs7095891 and 72 °C for 30 s and a final

Table 1 – Primer sequences of PCR-RFLP for detection of MBL2 polymorphisms

	Primer sequence (5'→3')	Restriction enzyme	Fragment (bp)
rs1800450 A>G	F: ATGGTGGCAGCGTCTTACTC R: TGGGCTGGCAAGACAACTAT	BanI	A allele: 346 G allele: 222+124
rs7095891	F: GTTAATCTCAGTTAATGAACACATATTTATC R: CCGAAAGCATGTTTATAGTCTTCCA	TaqI	A allele: 257 G allele: 226+31
rs777980157 (34bp ins/del)	F: CCTCCACGTGGCAACTTTATT R: TACCCGGACTTTTTCCAGGG		ins 313bp del 279bp

MBL2 – mannose-binding lectin 2

Taheri M.; Karimloo R.; Sarani H.; Molashahi B.; Naderi M.; Bahari G.; Hashemi M.

extension step of 72 °C for 5 min. Then, 10 μl of PCR product was digested with an appropriate restriction enzyme (Table 1). The PCR products were resolved on 2.5% agarose gel electrophoresis.

Statistical analysis
Data were analysed by the statistical package SPSS 20 software (SPSS for Windows, SPSS Inc., Illinois, USA) and the level of significance was set to a P-value of < 0.05. The differences between the variables were evaluated by chi-square test or independent sample *t*-test according to the data. The association between genotypes and PTB was assessed by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses.

Results
The study participants included 170 PTB patients (84 males and 86 females, mean age 51.31 ± 19.58) and 175 healthy controls (81 males and 94 females, mean age 49.86 ± 14.08). There was no difference in age and sex between the patient and healthy control groups (P=0.428 and 0.591, respectively). Table 2 reports the genotype and allele distribution of MBL2 rs1800450 and rs7095891 polymorphisms in cases and controls. Our data showed that GA genotype as well as GA+AA genotype of rs1800450 reduced the risk of PTB in comparison with GG genotype (OR=0.17, 95% CI=0.11–0.27, P<0.001; and OR=0.19, 95% CI=0.12–0.30, P<0.001, respectively). Further, A allele significantly decreased the risk of PTB (OR=0.36, 95% CI 0.25–0.52, P<0.001) in comparison with G allele. As listed in Table 2, the findings suggested that rs7095891 was associated with decreased risk of PTB in codominant (GA vs. GG: OR=0.118, 95% CI=0.054–0.258, P<0.001; and AA vs. GG: OR=0.029, 95% CI=0.01–0.082, P<0.001), dominant (GA+AA vs. GG: OR=0.095, 95% CI=0.044–0.207, P<0.001), and recessive (AA vs. GA+GG: OR=0.172, CI=0.081–0.365, P<0.001) inheritance models. The A allele can be considered as protective against PTB (OR=0.373, 95% CI=0.274–0.507, P<0.001) in comparison to G allele. The genotype frequency of rs777980157 polymorphism showed that all PTB patients and healthy controls had Ins/Ins genotype demonstrating that this variant is not polymorphic in our population.

Discussion
Tuberculosis is the second-leading cause of infectious diseases after HIV. Both genetic and environmental factors play an important role in influencing TB susceptibility. Innate immunity is the first line of defense against infection with Mtb. MBL2, is a calcium dependent plasma Collectin which binds to different microorganisms. It profoundly contributes to natural immune protection against infectious agents by activating the lactin complement pathway. It also regulates the inflammatory reactions. Many studies have demonstrated that MBL is involved in control of various microorganisms such as bacteria, fungi, parasites, and viruses

Table 2 – The genotypes and allele distribution of MBL2 polymorphisms in pulmonary tuberculosis (PTB) patients and control groups

	Patients n (%)	Normal n (%)	OR (95% CI)	P
rs1800450				
Codominant				
GG	121 (71)	56 (32)	1.00	
GA	43 (25)	116 (66)	0.172 (0.107–0.275)	<0.001
AA	6 (4)	3 (2)	2.2 (0.90–5.37)	0.080
Dominant				
GG	121 (71)	56 (32)	1.00	
GA+AA	49 (29)	119 (68)	0.191 (0.120–0.302)	<0.001
Recessive				
GG+GA	164 (96)	172 (98)	1.00	
AA	6 (4)	3 (2)	2.098 (0.516–8.526)	0.301
Alleles				
G	285 (84)	228 (65)	1.00	
A	55 (16)	122 (35)	0.360 (0.250–0.518)	<0.001
rs7095891				
Codominant				
GG	57 (34)	8 (4)	1.00	
GA	104 (61)	124 (71)	0.118 (0.054–0.258)	<0.001
AA	9 (5)	43 (25)	0.029 (0.01–0.082)	<0.001
Dominant				
GG	57 (34)	8 (4)	1.00	
GA+AA	113 (66)	167 (96)	0.095 (0.044–0.207)	<0.001
Recessive				
GG+GA	161 (95)	132 (75)	1.00	
AA	9 (5)	43 (25)	0.172 (0.081–0.365)	<0.001
Alleles				
G	218 (64)	140 (40)	1.00	
A	122 (36)	210 (60)	0.373 (0.274–0.507)	<0.001

MBL2 – mannose-binding lectin 2; OR – odds ratio; CI – confidence interval

(Eisen, 2010; Jha et al., 2014). In the current study, we aimed to find out the impact of MBL2 variants and risk of PTB in a sample of a southeast Iranian population. The results showed that GA as well as GA+AA genotype of rs1800450 reduced the risk of PTB. It was also found that rs7095891 variant significantly decreased the risk of PTB. Accordance to our results, Singla et al. (2012) found that mutant allele of rs1800450 has a protective role against TB, but they did not observe this result in extrapulmonary TB. Capparelli et al. (2009) in a study on Italian population illustrated that this polymorphism was protective against PTB in the heterozygote form and was more significantly protective in the homozygote form. When they did haplotype analysis, they found LYB/LYD haplotype increased the risk of tuberculosis

and concluded that MBL may be protective or risk factor of tuberculosis depending on host's haplotype pair (Capparelli et al., 2009). Cosar et al. (2008) discovered that mutant allele frequency of rs1800450 is significantly lower in the patient group. In contrast to this finding, Liu et al. (2016) revealed that subjects with variant allele of rs1800450 (homozygote and heterozygote) had an increased susceptibility to TB in comparison to wild type allele. Li et al. (2018) in a study on Chinese Uygur population found that MBL2 rs7095891 polymorphism was associated with an increased risk of TB; however, they did not find any association between rs1800450 as well as rs7096206 polymorphism and tuberculosis. Meanwhile, many studies have not found any association between MBL2 rs1800450 polymorphism and susceptibility or protection to tuberculosis (Soborg et al., 2007; Araújo et al., 2013; da Cruz et al., 2013; Wu et al., 2015; Amiri et al., 2017). The effect of MBL level on TB susceptibility has been controversial. Cosar et al. (2008) showed MBL plasma level was significantly lower in control groups in comparison with patients, while Capparelli et al. (2009) found that protection against TB was correlated with a high concentration of MBL in plasma. It has been shown that individuals with mutant genotype (0/0) have very low or undetectable levels of MBL (Frederiksen et al., 2006). Tong et al. (2019) in a meta-analysis found that MBL level in PTB patients was significantly lower than in the control group, implying that low expression of MBL2 is associated with an increased risk of PTB. These polymorphisms changed the structure of MBL proteins causing a functional deficiency and reduction of stability; as such they were more rapidly degraded and thus reduced the serum level of MBL (Heitzeneder et al., 2012; Mandal et al., 2019). Also, it seems that these MBL proteins have less binding capacity to mannose and cannot activate complement via the lectin pathway (Amiri et al., 2017). However, Thye et al. (2011) showed higher levels of MBL can promote infection by increasing the uptake of intracellular pathogens by phagocytes.

In conclusion, the present study revealed that rs1800450 and rs7095891 MBL2 polymorphisms reduced the risk of PTB in a sample of Iranian population. Replication in different ethnicities with more samples is required for better understanding of the association between these polymorphisms and risk of tuberculosis.

References

Amiri, A., Sabooteh, T., Shahsavar, F., Anbari, K., Pouremadi, F. (2017) Mannose-binding lectin (MBL) gene polymorphisms in susceptibility to pulmonary tuberculosis among the Lur population of Lorestan Province of Iran. *Genom. Data* **12**, 146–150.

Araújo, M. S., Graça, E. S., Azevedo, V. N., Cayres-Vallinoto, I., Machado, L. F. A., Ishak, M. O. G., Ishak, R., Vallinoto, A. C. R. (2013) No evidence of association between MBL2A/O polymorphisms and *Mycobacterium tuberculosis* infection in populations from the Brazilian Amazon region. *Hum. Immunol.* **74(1)**, 82–84.

Cao, Y., Wang, X., Cao, Z., Wu, C., Wu, D., Cheng, X. (2018) Genetic polymorphisms of MBL2 and tuberculosis susceptibility: a meta-analysis of 22 case-control studies. *Arch. Med. Sci.* **14(6)**, 1212–1232.

Capparelli, R., Iannaccone, M., Palumbo, D., Medaglia, C., Moscariello, E., Russo, A., Iannelli, D. (2009) Role played by human mannose-binding lectin polymorphisms in pulmonary tuberculosis. *J. Infect. Dis.* **199(5)**, 666–672.

Cosar, H., Ozkinay, F., Onay, H., Bayram, N., Bakiler, A. R., Anil, M., Can, D., Ozkinay, C. (2008) Low levels of mannose-binding lectin confers protection against tuberculosis in Turkish children. *Eur. J. Clin. Microbiol. Infect. Dis.* **27(12)**, 1165–1169.

da Cruz, H. L., da Silva, R. C., Segat, L., de Carvalho, M. S., Brandao, L. A., Guimaraes, R. L., Santos, F. C., de Lira, L. A., Montenegro, L. M., Schindler, H. C., Crovella, S. (2013) MBL2 gene polymorphisms and susceptibility to tuberculosis in a northeastern Brazilian population. *Infect. Genet. Evol.* **19**, 323–329.

Eisen, D. P. (2010) Mannose-binding lectin deficiency and respiratory tract infection. *J. Innate Immun.* **2(2)**, 114–122.

Frederiksen, P. D., Thiel, S., Jensen, L., Hansen, A. G., Matthiesen, F., Jensenius, J. C. (2006) Quantification of mannan-binding lectin. *J. Immunol. Methods* **315(1–2)**, 49–60.

Guo, Y. L., Liu, Y., Ban, W. J., Sun, Q., Shi, G. L. (2017) Association of mannose-binding lectin gene polymorphism with the development of pulmonary tuberculosis in China. *BMC Infect. Dis.* **17(1)**, 210.

Hashemi, M., Sharifi-Mood, B., Rasouli, A., Amininia, S., Naderi, M., Taheri, M. (2015) Macrophage migration inhibitory factor −173 G/C polymorphism is associated with an increased risk of pulmonary tuberculosis in Zahedan, Southeast Iran. *EXCLI J.* **14(21)**, 117–122.

Heitzeneder, S., Seidel, M., Forster-Waldl, E., Heitger, A. (2012) Mannan-binding lectin deficiency – Good news, bad news, doesn't matter? *Clin. Immunol.* **143(1)**, 22–38.

Huang, X., Yang, Y., Cui, Z. W., Wang, J., Gao, L. B. (2016) A functional insertion/deletion polymorphism in the IL1A gene is associated with decreased risk of breast cancer. *Genet. Mol. Res.* **15(1)**, 26909967.

Jha, A. N., Sundaravadivel, P., Singh, V. K., Pati, S. S., Patra, P. K., Kremsner, P. G., Velavan, T. P., Singh, L., Thangaraj, K. (2014) MBL2 variations and malaria susceptibility in Indian populations. *Infect. Immun.* **82(1)**, 52–61.

Kouhpayeh, H. R., Hashemi, M., Hashemi, S. A., Moazeni-Roodi, A., Naderi, M., Sharifi-Mood, B., Taheri, M., Mohammadi, M., Ghavami, S. (2012) R620W functional polymorphism of protein tyrosine phosphatase non-receptor type 22 is not associated with pulmonary tuberculosis in Zahedan, southeast Iran. *Genet. Mol. Res.* **11(2)**, 1075–1081.

Kouhpayeh, H. R., Taheri, M., Baziboroon, M., Naderi, M., Bahari, G., Hashemi, M. (2016) CCL5 rs2107538 polymorphism increased the risk of tuberculosis in a sample of Iranian population. *Prague Med. Rep.* **117(2–3)**, 90–97.

Li, X., Cao, X., El-Ashram, S., Zhang, W., Lu, L., Wang, X., Chen, C., Wu, J. (2018) MBL2 rs7095891 G > A polymorphism was associated with an increased risk of tuberculosis in the Chinese Uygur population. *Int. J. Mol. Epidemiol. Genet.* **9(5)**, 64–70.

Liu, C., He, T., Rong, Y., Du, F., Ma, D., Wei, Y., Mei, Z., Wang, Y., Wang, H., Zhu, Y., Zhang, Z., Zheng, L., Wu, X., Liu, H., Ding, W. (2016) Association of mannose-binding lectin polymorphisms with tuberculosis susceptibility among Chinese. *Sci. Rep.* **6**, 36488.

Mandal, R. K., Khan, M. A., Hussain, A., Dar, S. A., Aloufi, S., Jawed, A., Wahid, M., Panda, A. K., Lohani, M., Akhter, N., Khan, S., Mishra, B. N., Haque, S. (2019) Association of MBL2 gene polymorphisms with pulmonary tuberculosis susceptibility: trial sequence meta-analysis as evidence. *Infect. Drug Resist.* **12**, 185–210.

Metanat, M., Sharifi-Mood, B., Shahreki, S., Dawoudi, S. H. (2012) Prevalence of multidrug-resistant and extensively drug-resistant tuberculosis in patients with pulmonary tuberculosis in Zahedan, southeastern Iran. *Iran. Red Crescent Med. J.* **14(1)**, 53–55.

Singla, N., Gupta, D., Joshi, A., Batra, N., Singh, J., Birbian, N. (2012) Association of mannose-binding lectin gene polymorphism with tuberculosis susceptibility and sputum conversion time. *Int. J. Immunogenet*. **39(1)**, 10–14.

Soborg, C., Andersen, A. B., Range, N., Malenganisho, W., Friis, H., Magnussen, P., Temu, M. M., Changalucha, J., Madsen, H. O., Garred, P. (2007) Influence of candidate susceptibility genes on tuberculosis in a high endemic region. *Mol. Immunol*. **44(9)**, 2213–2220.

Thye, T., Niemann, S., Walter, K., Homolka, S., Intemann, C. D., Chinbuah, M. A., Enimil, A., Gyapong, J., Osei, I., Owusu-Dabo, E., Rusch-Gerdes, S., Horstmann, R. D., Ehlers, S., Meyer, C. G. (2011) Variant G57E of mannose binding lectin associated with protection against tuberculosis caused by *Mycobacterium africanum* but not by *M. tuberculosis*. *PLoS One* **6(6)**, e20908.

Tong, X., Wan, Q., Li, Z., Liu, S., Huang, J., Wu, M., Fan, H. (2019) Association between the mannose-binding lectin (MBL)-2 gene variants and serum MBL with pulmonary tuberculosis: An update meta-analysis and systematic review. *Microb. Pathog*. **132**, 374–380.

World Health Organization (2019) *Global Tuberculosis Report*. Available at: https://apps.who.int/iris/bitstream /handle/10665/329368/9789241565714-eng.pdf?ua=1

Wu, L., Deng, H., Zheng, Y., Mansjo, M., Zheng, X., Hu, Y., Xu, B. (2015) An association study of NRAMP1, VDR, MBL and their interaction with the susceptibility to tuberculosis in a Chinese population. *Int. J. Infect. Dis*. **38**, 129–135.

Zheng, M., Shi, S., Wei, W., Zheng, Q., Wang, Y., Ying, X., Lu, D. (2018) Correlation between MBL2/CD14/ TNF-alpha gene polymorphisms and susceptibility to spinal tuberculosis in Chinese population. *Biosci. Rep*. **38(1)**, BSR20171140.

Zhou, X., Zhou, Q., Yang, Z. F., Li, W. X. (2018) Genetic polymorphism of human leucocyte antigen and susceptibility to multidrug-resistant and rifampicin-resistant tuberculosis in Han Chinese from Hubei Province. *Int. J. Immunogenet*. **45(1)**, 8–21.