Correlation between Vitamin D Receptor Gene FOKI and BSMI Polymorphisms and the Susceptibility to Pulmonary Tuberculosis in an Indonesian Batak-ethnic Population

Bintang Y.M. Sinaga[1], Muhammad Amin[2], Yahwardiah Siregar[3], Sori M. Sarumpaet[4]

[1] Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Sumatera Utara - Adam Malik Hospital, Medan, Indonesia.
[2] Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga – dr. Soetomo Hospital, Surabaya, Indonesia.
[3] Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.
[4] Department of Epidemiology, Faculty of Public Health, Universitas Sumatera Utara, Medan, Indonesia.

Correspondence mail:
Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Sumatera Utara - Adam Malik Hospital. Jl. Bunga Lau no. 17, Medan, Sumatera Utara, Indonesia. email: bymsinaga@yahoo.co.id.

ABSTRAK

Tujuan: *untuk mengetahui peran polimorfisme FokI dan BsmI gen reseptor vitamin D (RVD) pada kerentanan terhadap tuberkulosis (TB) paru suku Batak di Indonesia.* ***Metode:*** *disain penelitian adalah kasus kontrol berpasangan, dengan 76 pasien TB paru sebagai kasus dan 76 orang sehat yang tidak menderita TB paru sebagai kontrol. Polimorfisme gen RVD dianalisis menggunakan teknik PCR-RFLP.* ***Hasil:*** *frekuensi genotip FokI adalah FF 35.5%, Ff 55.3%, ff 9.2% pada kelompok TB paru dan FF 39.5%, Ff 44.7.%, 15.8% pada kelompok kontrol. Frekuensi genotip BsmI adalah BB 0%, Bb 68.4%, bb 31.6% pada kelompok TB paru dan BB 2.6%, Bb 23.7% and bb 73.7% pada kelompok kontrol. Tidak ada hubungan yang bermakna antara genotip FokI dan kerentanan TB paru (OR 1.39, 95% CI: 0.69-2.77 untuk genotip Ff dan OR 0.64, 95% CI: 0.22-1.86 untuk genotip ff). Pada polimorfisme BsmI, ada hubungan yang bermakna antara genotip BsmI dan kerentanan terhadap TB paru. Genotip bb berhubungan dengan penurunan risiko terhadap terjadinya TB paru (OR 0.22, 95% CI: 0.11-0.45).* ***Kesimpulan:*** *pada populasi Indonesia etnik Batak, tidak ada hubungan antara polimorfisme FokI gen RVD dan kerentanan terhadap TB paru. Pada polimorfisme BsmI gen RVD, genotip bb berhubungan dengan penurunan risiko terhadap TB paru.*

Kata kunci: *tuberkulosis paru, polimorfisme, gen reseptor vitamin D, Batak, Indonesia.*

ABSTRACT

Aim: *to explore the role of FokI and BsmI polymorphisms the VDR gene in the susceptibility to pulmonary tuberculosis (PTB) in an Indonesian Batak ethnic population.* ***Methods:*** *matched case-control study was conducted on 76 PTB patients and 76 healthy normal control. Genetic polymorphisms of Vitamin D Receptor (VDR) gene were analysed using PCR-RFLP.* ***Results:*** *the frequencies of FokI genotypes were FF 35.5%, Ff 55.3%, ff 9.2% for PTB patients and FF 39.5%, Ff 44.7.% and ff 15.8% for normal control. The BsmI genotypes frequencies were BB 0%, Bb 68.4%, bb 31.6% for TB patients and BB 2.6%, Bb 23.7% and bb 73.7% for control. There was no significant association between FokI genotype and PTB (OR 1.39, 95% CI: 0.69-2.77 for Ff genotype and OR 0.64, 95% CI: 0.22-1.86 for ff genotype). There was a significant association between BsmI*

genotype and PTB; the bb genotype was associated with a decreased risk to PTB (OR 0.22, 95% CI: 0.11-0.45). ***Conclusion:*** *in Indonesian Batak ethnic population, there was no association between FokI polymorphism of VDR gene with host susceptibility to PTB. There was a significant association between BsmI polymorphism of VDR gene; bb genotype was associated with a decreased risk to PTB.*

Key words: *pulmonary tuberculosis, polymorphisms, vitamin D receptor gene, Batak, Indonesia.*

INTRODUCTION

Pulmonary tuberculosis (PTB) is still a public health problem in the world. About 1/3 of people in the world are infected with Mycobacterium tuberculosis, but only 10% of them develop clinical disease. Susceptibility to disease after mycobacteria infection is influenced by agent, environmental and host genetic factors.[1,2] Many studies have strongly indicated that genetic factor plays an important role in the disease development. Vitamin D receptor gene is one of the recently interesting candidate gene.

Beside a calcium, phosphor and bone metabolism regulator, vitamin D also has nonskeletal function. Many studies showed an association between vitamin D deficiency and certain diseases such as systemic lupus erythematosis,[3] diabetes mellitus,[4] tuberculosis,[5] etc. In tuberculosis infection, vitamin D can activate macrophage to restrict *Mycobacterium tuberculosis* intracellular growth. This effect is achieved through binding to vitamin D receptor (VDR) in macrophages, hence activate cathelicidin synthesis,[6] and consequently eliminate Mycobacterium tuberculosis in phagolysosomes.[7] Those process might be affected by polymorphisms in the VDR gene.

One of these polymorphisms is FokI, transition C to T (ACG-ATG) at the first of the two potential translation initiation sites in exon 2, and can be distinguised by RFLP using endonuclease FokI. If translation start at the first ATG site (individuals with T allele, designated f), VDR protein synthesized full-length (427 amino acids). In contrast, if the translation start at the second ATG site (individuals with C allele, designated F), the VDR protein lack the three NH2-terminal amino acids.[8] Some studies indicated that transcription of the F allele is 1.7 more than f allele,[9] F allele interacts with transcription factor IIB more efficiently and result in more potent VDR protein transcription.[10] The second polymorphism in this study is BsmI, which located at intron between exon VIII and IX. The BsmI polymorphism influence transcription level, transcription stability and post modification transcription of VDR gene.[11]

The potential roles of VDR FokI and BsmI polymorphisms in the development of PTB have been investigated in many ethnic group. The different results may be due to diverse ethnic background. Meta-analysis by Gao (2010) found that in Asians, subjects with ff genotype were more susceptible to TB and subjects with bb genotype have a decrease risk for TB. However, none of the polymorphisms was significantly related to TB among Africans or South Americans.[12] Because the genetic effect may be different in various ethnic group, we undertook this study in Indonesian Batak-ethnic population, to know the role of FokI and BsmI polymorphisms VDR gene in the susceptibility to pulmonary tuberculosis.

METHODS

This is a matched case-control study with FokI and BsmI vitamin D receptor gene polymorphisms as independent variable and pulmonary tuberculosis as dependent variable. Sample size was determined by using matched case-control formula and number of sample was 76 subjects for each group. Cases were found by consecutive sampling and controls were subjects from healthy workers. This study has been approved by the Ethics Committee of the Faculty of Medicine, University of Sumatera Utara, Medan, Indonesia.

Subjects

Cases were pulmonary tuberculosis patients were recruited from several TB services in Medan city, Indonesia, from November 2012 to April

2013. The inclusion criteria in the case group were newly diagnosed pulmonary tuberculosis patients, age 16-55 years old, Batak ethnic, have symptoms of pulmonary TB, positive sputum smear and chest radiography consistent with active disease. The exclusion criteria in case group were HIV positive, known to present diabetes mellitus and other severe diseases, and consuming imunnosupressive drugs.

Control group composed of sex, age and ethnically matched healthy subjects with normal chest X-ray and no history of previous tuberculosis. They were healthy workers like doctors, nurses and medical students. Tuberculin test was done in the control group in which 47 subjects (61.7%) was positive to the test.

All subjects were interviewed and informed consent was obtained. An anticoagulated peripheral blood specimen was collected and polymorphisms of Vitamin D Receptor (VDR) gene were analysed using PCR-RFLP.

VDR Genotyping

The DNA was extracted (Promega, USA) and stored at minus 20^0C. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) was used to identify FokI and BsmI polymorphism of Vitamin D Receptor gene. The primer sequences used in this study were as follows: Forward Primer: 5'-AGC TGG CCC TGG CAC TGA CTC TGC TCT-3' and Reverse Primer: 5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3' for FokI. Forward Primer 5'CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA-3' and Reverse Primer: 5'AAC CAG CGG GAA GAG GTC AAG GG-3' for BsmI.

PCR conditions were as follows: denaturation at 94^0C for 5 min, followed by 35 cycles of PCR at 94^0C (30 sec), annealing at 61^0C for FokI and BsmI (30 sec), and 72^0C (1 min). Final extension was continued at 72^0C for 7 min. Following PCR, the amplified PCR products was digested with FokI (Takara, Bio-Inc, Japan) restriction enzyme at 37^0C for 3 hours and BsmI (Thermo Scientific, Lithuania) restriction enzyme at 37^0C for 4 hours. Digested products were analyzed using electrophoresis in 2% agarose gel and ethidium bromide stains. The bands were visualyzed by Gel Documentation System.

Depending on the digestion pattern of FokI polymorphism, individuals were scored as ff when homozygous for the presence of the FokI site (169 bp and 96 bp), FF when homozygous for the absence of the FokI site (265 bp), or Ff in case of heterozygosity (265 bp, 169 bp and 96 bp). The digestion pattern of BsmI polymorphism were bb for the presence of BsmI site (175 and 650 bp), BB when homozygous for the absence of the BsmI site (825 bp), and Bb in case of heterozygote (825, 175 and 650 bp).

Statistical Analysis

The genotype frequencies of each SNPs were compared by Mc Nemar Chi-square test as appropriate. The strength of the association between VDR FokI, BsmI polymorphisms and TB risk was evaluated by calculating odds ratio (OR) with 95% confidence interval (95% CI). Conditional logistic regression was performed to calculate the odds ratio. Data were managed and analysed using Epi Info. Hardy-Weinberg equilibrium test was done in case and control groups for FokI and BsmI polymorphisms using the web tool HWE Testing calculator, available on line[13]; $p<0.05$ was considered as a significant disequilibrium.

RESULTS

The characteristics of PTB patients and normal control are summarized in **Table 1**. Sex, age and ethnic characteristics between PTB patients and controls were matched.

The results of VDR genotyping for PTB patients and healthy controls are summarized in **Table 2**. Pulmonary tuberculosis patients and

Table 1. Characteristic of PTB cases and controls group

Characteristic	Cases - n (%)	Control - n (%)
Sex		
- Male	53 (69.7)	53 (69.7)
- Female	23 (30.3)	23 (30.3)
Age		
- 16-25	22 (28.9)	22 (28.9)
- 26-35	32 (42.1)	32 (42.1)
- 36-45	14 (18.4)	14 (18.4)
- 46-55	8 (10.5)	8 (10.5)
Ethnicity		
- Batak ethnic	76 (100)	76 (100)

healthy controls had similar distribution for FokI genotype and allele frequencies (p>0.05). On the BsmI genotype and allele frequencies, there was a significant difference between PTB patients and healthy controls (p<0.001). The genotypes of FokI polymorphism in cases and controls group were in Hardy-Weinberg equilibrium (P>0.05) and BsmI polymorphism in controls group as well. The BsmI polymorphism in cases group was not in Hardy-Weinberg equilibrium (p<0.05).

Relation between FokI and BsmI polymorphisms and pulmonary tuberculosis are summarized in **Table 3**. There was no significant association between FokI polymorphisms and PTB (OR 1.39, 95% CI: 0.69-2.77 for Ff genotype and OR 0.64, 95% CI: 0.22-1.86 for ff genotype). On the BsmI polymorphism, the bb genotype was associated with a decreased risk to PTB (OR 0.22, 95% CI: 0.11-0.45).

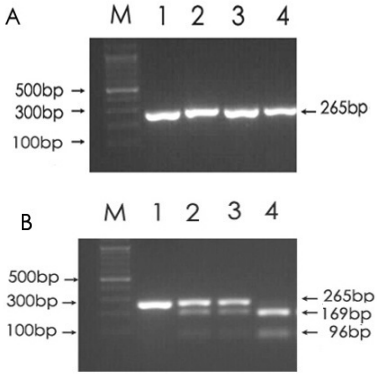

Figure 1. FokI restriction patterns of various genotype. A. PCR product before treatment with restriction enzyme. B. Fragments after restriction enzyme treatment. The FF genotype (B1) had one band at 265 bp, the ff genotype (B4) had 2 bands at 169 and 96 bp, and the Ff heterozygous genotype (B2 and B3) had three bands at 265 bp, 169 bp and 96 bp.

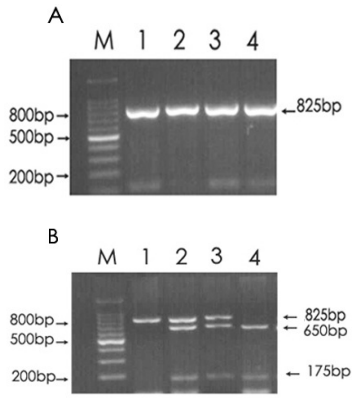

Figure 2. BsmI restriction patterns of various genotype. A. PCR product before treatment with restriction enzyme. B. Fragments after restriction enzyme treatment. The BB genotype (B1) had one band at 825 bp, the bb genotype (B4) had 2 bands at 650 and 175 bp, and the Bb heterozygous genotype (B2 and B3) had three bands at 825 bp, 650 bp and 175 bp.

Table 2. Allele frequencies and genotype of VDR gene FokI and BsmI polymorphisms in PTB cases and controls

Polymorphism	Cases n (%)	Controls n (%)	p	HWE in cases X^2 (p)	HWE in controls X^2 (p)
FokI					
Genotype FF	27 (35.5)	30 (39.5)	0.311	2.67 (>0.05)	0.21 (>0.05)
Ff	42 (55.3)	34 (44.7)			
ff	7 (9.2)	12 (15.8)			
Total	76 (100)	76 (100)			
Allele frequencies					
F	96 (63.2)	94 (61.8)	0.816		
f	56 (36.8)	58 (38.2)			
Total	152 (100)	152 (100)			
BsmI					
Genotype BB	0	2 (2.6)	<0.001	20.55 (<0.05)	0.14 (>0.05)
Bb	52 (68.4)	18 (23.7)			
bb	24 (31.6)	56 (73.7)			
Total	76 (100)	76 (100)			
Allele frequencies					
B	52 (34.2)	22 (14.5)	<0.001		
b	100 (65.8)	130 (85.5)			
Total	152 (100)	152 (100)			

HWE = Hardy- Weinberg Equilibrium; p<0.05 was considered as significant disequilibrium.

Table 3. Analysis of VDR gene FokI and BsmI polymorphisms in PTB cases and controls

Polymorphism	Cases n (%)	Control n (%)	OR (95% CI)	p
FokI				
Genotype FF	27 (35.5)	30 (39.5)	1	
Ff	42 (55.3)	34 (44.7)	1.39 (0.69– 2.77)*	0.352
ff	7 (9.2)	12 (15.8)	0.64 (0.22 – 1.86)*	0.418
BsmI				
Genotype Bb+Bb	52 (68.4)	20 (26.3)	1	
bb	24(31.6)	56 (73.7)	0.22 (0.11 - 0.45)*	<0.001

*Odds ratio was calculated using conditional logistic regression analysis

DISCUSSION

Susceptibility to TB is the complex interaction between host, bacteria (agent) and environment. Host genetics is one of the host factor that can influence this susceptibility.

Comparisons with other researches showed that population or various ethnicities give different results. The non-significant relationship between VDR FokI polymorphism and PTB in this study were also found in Korea,[14] Iran,[15] Africa,[16-19] South American,[20] and spinal TB in India.[21] Association between ff genotype FokI polymorphism and susceptibility to PTB has been found in Han population in China,[22] and association between FF genotype and susceptibility to PTB was found in Indian males.[23] A meta-analysis in China on Han ethnic showed an association between ff genotype and susceptibilty to PTB.[24] Other meta-analysis from 13 studies showed association between ff genotype and susceptibility to PTB[25] as well, and another meta-analysis from 29 case-control studies showed association between ff genotype and susceptibility to PTB in Chinese but not for other ethnicities.[26] Another meta-analysis showed that ff genotype was associated with TB susceptibility among Asian population.[12]

Studies from Africa,[17,27] India,[28] and Korea[14] found no association between BsmI polymorphism of VDR gene and PTB. The bb genotype was protective factor to PTB and extra PTB in Turki.[29] Studies from Iran found bb genotype[16] and Bb+bb genotype[30] was associated with susceptibility to PTB. Meta-analysis from 15 studies found b allele and bb genotype associated with decreased risk to TB, especially in Asian population.[31] In this study, we found that bb genotype was a protective factor to PTB or associated with decreased risk to PTB.

Some explanations are possible for the different results of this study compared to others. Ethnicity factor plays an important role. This study was restricted to Batak ethnic Indonesian population only, to prevent genetic bias from ethnic influence. Ethnicity is found to be a very important factor on genetic function in pulmonary TB disease. This can be seen in the different distribution of FokI allele in the world among populations or ethnics. Frequency of f allele is lower among African race (24%) compared to the Caucasians (34%) and Asians (51%). For BsmI polymorphism, the frequency of B allele is 7% among Asians, 36% among Africans and 42% among Caucasians.[32] Another studies found the BsmI bb genotype frequency was 2% among Asians, 5% among Africa Americans and 17% among Caucasians.[8]

The difference in the definition of case and control in each study can also alter the results. Some studies presume the case group as negative acid fast bacilli bacteria sputum or extra pulmonary TB, whereas for the control group, some studies took blood samples from donor banks where history of exposure to TB is not known. Similarly, not all studies have conducted HIV tests to determine the HIV status of the subjects.[33]

The different results could also be caused by gene-environment interaction, gene-gene interaction and gene-agent interaction. A study on Indian Gujarat ethnic resides in London showed no association between FokI polymorphism with

TB, but together with vitamin D deficiency, ff genotype is associated with susceptibility to TB. This study confirm gene-environment interaction.[34] Gene-gene interaction has been shown in some studies. A single gene that is not associated with susceptibility to TB will show an association if combined with other genes.[35,36] Gene-agent interaction showed association of a certain host gene with a certain strain of Mycobacterium tuberculosis.[37,38]

The latest study showed an epigenetic variation on VDR gene and this discrepancy is different among various ethnic groups. Epigenetic variation and VDR TaqI polymorphism interrelate with TB susceptibility. This demonstrates that interaction of genetic variation (genotype) with epigenetic variation (epigenotype) must be considered for associated study on diverse population.[39]

There are other factors that may influence the results. It is known that before vitamin D enters the macrophage, inactive vitamin D in serum could be bound to vitamin D binding protein (DBP) or in a free state. After binding to Toll-Like Receptor on macrophage, inactive vitamin D is converted to active form by CYP27b1 enzyme on mitochondria. Consequently, along with heat shock protein 70 (hsc70) and bcl-2 associated athanogene (BAG-1), active vitamin D and its receptor enter the nucleus and forms heterodimer with Retinoid X Receptor (RXR) henced bind to Vitamin D Response Elements (VDRE) which is controlled by vitamin D response elements – binding protein (VDRE-BP) on gene target promoter of cathelicidin.[7] Therefore, all that occur in transcription of cathelicidin can influence the results of the study.

Further studies are needed to know the role of Vitamin D Receptor in susceptibility to TB. In order to understand the pathogenesis of pulmonary TB that is useful for therapy and prophylaxis. Ethnic-specific genetic associated with TB susceptibility may guide TB therapy and prophylaxis in an ethnic-specific manner.

CONCLUSION

In this study which is conducted in an Indonesian Batak ethnic population, FokI polymorphism of VDR gene is not responsible for host susceptibility to PTB. On the BsmI polymorphism, bb genotype was associated with decreased risk to PTB. These results, particularly for bb genotype BsmI polymorphisms, may give additional explanation why some people are more resistant against TB than the others. On the other hand, persons whose genotype that may cause them to be more susceptible to TB should be more aware about things that influence their immunity like nutrition, lifestyle such as smoking, alcohol consuming, etc.

ACKNOWLEDGMENTS

This study was partly supported by Directorate General of Higher Education, Ministry of Education and Culture, Indonesia (Hibah Bersaing Scheme; No. 4267/UN5.1.R/KEU/2013).

REFERENCES

1. Bellamy R, Ruwende C, Corrag T, et al. Variations in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. N Engl J Med. 1998;338:640-4.
2. Buschman E, Skamene E, Schurr E. The role of the NRAMP1 gene in mycobacterial infection. In: Rom WN, Garay SM, eds. Tuberculosis. Philadelphia: Lippincott William & Wilkins; 2004. p. 193-201.
3. Handono K, Marisa D, Kalim H. Association between the low levels of vitamin D and treg function in systemic lupus erythematosus patients. Acta Med Indones. 2013;45(1):26-31.
4. Afzal S, Bojesen SE, Nordestgaard BG. Low 25-Hydroxyvitamin D and risk of type 2 diabetes: A prospective cohort study and metaanalysis. Clin Chem. 2013;59(2):381-91.
5. Nnoaham KE, Clarke A. Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. Int J Epidemiol. 2008;37:113-9.
6. Liu PT, Stenger S, Tang DH, et al. Cutting edge: Vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. J Immunol. 2007;179: 2060-3.
7. Chun RF, Adams JS, Hewison M. Back to the future: a new look at 'old' vitamin D. J Endocrinol. 2008;198:261-9.
8. Zmuda JM, Cauley JA, Ferrell RE. Molecular epidemiology of vitamin D receptor gene variants. Epidemiol Rev. 2000;22:203-17.
9. Arai H, Myamoto K, Taketani Y, et al. A vitamin D receptor gene polymorphism in the tranlation initiation codon: effect on protein and relation to bone mineral density in Japanese women. JBMR. 1997;12:915-21.

10. Jurutka PW, Remus LS, Whitfield GK, et al. The polymorphic N terminus in human vitamin D receptor isoform influences transcriptional activity by modulating interaction with transcription factor IIB. Mol Endocrinol. 2000;14:401-20.
11. Kaleta B, Bogaczewicz J, Robak E, et al. Vitamin D Receptor gene BsmI polymorphism in Polish patients with systemic lupus erythematosus. ISRN Endocrinol. 2013 doi 10.1155/2013/427818.
12. Gao L, Tao Y, Zhang L, et al. Vitamin D receptor genetic polymorphisms and tuberculosis: updated systematic review and meta-analysis. Int J Tuberc Lung Dis. 2010;14:15-23.
13. Rodriguez S, Gaunt TR, Day lan NM. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Available at http://www.oege.org/software/hwe-mr-calc.html. Access on May 27, 2014.
14. Kang TJ, Jin SH, Yeum C, et al. Vitamin D receptor gene TaqI, BsmI and FokI polymorphisms in Korean patients with tuberculosis. Immune Netw. 2011;11:253-7.
15. Banoei MM, Mirsaeidi MS, Houshmand M, et al. Vitamin D receptor homoxygote mutant tt and bb are associated with susceptibility to pulmonary tuberculosis in the Iranian population. Int J Infect Dis. 2010;14:e84-e85.
16. Bornman L, Campbell SJ, Fielding K, et al. Vitamin D receptor polymorphisms and susceptibility to tuberculosis in West Africa: A case-control and family study. J Infect Dis. 2004;190:1631-41.
17. Lombard Z, Dalton DL, Venter PA, et al. Association of HLA-DR, -DQ and vitamin D receptor alleles and haplotypes with tuberculosis in the Venda of South Africa. Hum Immunol. 2006;67:643-54.
18. Babb C, van der Merwe L, Beyers N, et al. Vitamin D receptor gene polymorphisms and sputum conversion time in pulmonanary tuberculosis patients. Tuberculosis (Edinb). 2007;87:295-302.
19. Olesen R, Wejse C, Velez DR, et al. DC-SIGN (CD209), pentraxin 3 and vitamin D receptor gene variants associate with pulmonary tuberculosis risk in West Africans. Genes Immun. 2007;8:456-67.
20. Wilbur AK, Kubatko LS, Hurtado AM, et al. Vitamin D receptor gene polymorphisms and susceptibility M. tuberculosis in Native Paraguayans. Tuberculosis. 2007;87:329-37.
21. Selvaraj P, Kurian SM, Chandra G, et al. Vitamin D receptor gene variants of BsmI, ApaI, TaqI, and FokI polymorphisms in spinal tuberculosis. Clin Genet. 2004;65:73-6.
22. Liu W, Cao W, Zhang C, et al. VDR and NRAMP1 gene polymorphisms in susceptibility to pulmonary tuberculosis among the Chinese Han population: a case-control study. Int J Tuberc Lung Dis. 2004;8:428-34.
23. Selvaraj P, Chandra G, Kurian SM, et al. Association of vitamin D receptor gene variants of BsmI, ApaI and FokI polymorphisms with susceptibility or resistance to pulmonary tuberculosis. Curr Sci. 2003;84:1564-8.
24. Shang CAO, Peng-fei LUO, Wei LI, et al. Meta analysis. Vitamin D receptor genetic polymorphisms and tuberculosis among Chinese Han ethnic group. Chin Med J. 2012;125:920-5.
25. Qian S, Xiangyu M, Hui L, et al. Association between gene polymorphisms of vitamin D receptor and pulmonary tuberculosis susceptibility: a meta-analysis. Journal of medical colleges of PLA. 2011;26:67-75.
26. Chen C, Liu Q, Zhu L, et al. Vitamin D receptor gene polymorphisms on the risk of tuberculosis, a meta-analysis of 29 case-control studies. PloS ONE. 8(12): e83843. doi:10.1371/journal.pone.0083843.
27. Fitness J, Floyd S, Wandorff DK, et al. Large scale candidate gene study of tuberculosis susceptibility in Karonga district of Northern Malawi. Am J Trop Med Hyg. 2004;71:341-9.
28. Shing A, Gaughan JP, Kashyap VK. SLC11A1 and VDR gene variants and susceptibility to tuberculosis and disease progression in East India. Int J Tuberc Lung Dis. 2011;15:1468-4.
29. Ates O, Dolek B, Dalyan L, et al. The association between BsmI variant of vitamin D receptor gene and susceptibility to tuberculosis. Mol Biol Rep. 2011;38: 2633-6.
30. Merza M, Farnia P, Anoosheh S, et al. The NRAMP1, VDR and TNF-α gene polymorphisms in Iranian tuberculosis patients: the study on host susceptibility. Braz J Infect Dis. 2009;13:252-6.
31. Wu Y, Yang X, Wang X, et al. Association of vitamin D receptor Bsm I gene polymorphism with risk of tuberculosis: A Meta-analysis of 15 studies. PloS ONE. 2010;8(6):e66944. doi: 10.1371/journal.pone.0066944.
32. Uitterlinden AG, Fanf Y, van Meurs JBJ, et al. Genetics and biology of vitamin D receptor polymorphisms. Gene. 2004;338:143-56.
33. Stein CM. Genetic epidemiology of tuberculosis susceptibility: impact of study design. PLoS Pathogens.2011; 7(1):e1001189. doi:10.1371/jornal.ppat.1001189.
34. Wilkinson RJ, Liewelyn M, Toossi Z, et al. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in West London: a case-control study. Lancet. 2000;355:618-21.
35. Velez DR, Hulme WF, Myers JL, et al. NOS2A, TLR4, and IFNGR1 interaction influence pulmonary tuberculosis susceptibility in African-Americans. Hum Genet. 2009;126:643-53. doi:10.1007/s00439-009-0713-y.
36. Ben-Selma W, Harizi H, Boukadida J. Association of TNF-α and IL-10 polymorphisms with tuberculosis in Tunisian populations. Microbes Infect 2011;13:837-43.
37. Takahashi K, Hasegawa Y, Abe T, et al. SLC11A1 (formerly NRAMP1 polymorphisms associated with multidrug-resistant tuberculosis. Tuberculosis. 2008;88:52-7.

38. Van Crevel R, Parwati I, Sahiratmadja E, et al. Infection with Mycobacterium tuberculosis Beijing genotype strains is associated with polymorphisms in SLC11A1/NRAMP1 in Indonesian patients with tuberculosis. J Infect Dis. 2009;200:1671-4.
39. Andraos C, Koorsen G, Knight JC, et al. Vitamin D receptor gene methylation is associated with ethnicity, tuberculosis, and TaqI polymorphism. Hum Immunol. 2010;72:262-8.