International Journal of Infectious Diseases (2010) **14**, e84—e85

http://intl.elsevierhealth.com/journals/ijid

LETTER TO THE EDITOR

Vitamin D receptor homozygote mutant *tt* and *bb* are associated with susceptibility to pulmonary tuberculosis in the Iranian population

Tuberculosis (TB) is one of the largest single infectious causes of death worldwide.[1] Susceptibility to TB is influenced by host genetic factors.[2] The vitamin D receptor (VDR) is an area of interest related to genetic polymorphisms associated with TB.

The active metabolite of vitamin D, 1,25-dihydroxyvitamin D_3, is an important immunomodulatory hormone that enhances phagocytosis via the activation of macrophages. Vitamin D_3 has also been shown to restrict the growth of *Mycobacterium tuberculosis* in macrophages.[3] Recent studies have indicated many polymorphisms to exist in the vitamin D receptor (VDR) gene, but the influence of VDR gene polymorphisms on VDR protein function and signaling is largely unknown. So far, three adjacent restriction fragment length polymorphisms for *Fok*I, *Bsm*I, and *Taq*I, at the VDR gene have been studied most frequently. *Fok*I (424 and 427 amino acid variants) appears to be functional, and the 424aa variant is more active in terms of transactivation capacity. However, *Bsm*I and *Taq*I are in linkage disequilibrium with the 3′-untranslated region (3′UTR) and functionality is currently assumed to be due to this.[4]

Although the role of VDR polymorphisms in TB has been evaluated in several studies, results have been inconsistent. In order to gain a better understanding of the role of VDR polymorphism in TB susceptibility, we examined the polymorphisms of the VDR gene in Iranian TB patients.

A total of 60 unrelated patients of Iranian ancestry with confirmed pulmonary TB (PTB) were evaluated in this study. Patients were enrolled from Massih Daneshvari Hospital, a TB referral center in Iran, from February to August 2005. A control group consisted of 62 healthy subjects selected randomly from blood donor centers close to the reported residences of the 60 cases.

Exclusion criteria for both case and control groups were: concurrent disease predisposing to TB (e.g., HIV/AIDS, diabetes mellitus, liver cirrhosis, chronic renal failure), use of immunosuppressive drugs and organ transplantation, and malignancy with the exception of basal cell carcinoma. Additionally, potential controls were excluded if the patient had a past history of TB. In agreement with the protocols approved by the Institutional Review Board, written consent was obtained from all participants.

For each patient, 10 ml of blood was drawn and stored. VDR genotyping of *Fok*I, *Bsm*I, and *Taq*I polymorphisms was carried out by PCR amplification using the genomic DNA of the patients and normal subjects and specific primers as previously described.[5] All data were statistically analyzed with SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

The mean $\pm$ standard deviation age of the TB group was 45.8 ± 11 years and the control group was 41 ± 9 years ($p = 0.74$); 30 (50%) of the TB group and 36 (58%) of the control subjects were male ($p = 0.38$).

Table 1 summarizes allele and genotype frequencies of *Fok*I, *Bsm*I, and *Taq*I polymorphisms of VDR pulmonary TB and control subjects.

The main findings of our study are: (1) the *Fok*I locus of the VDR gene is not associated with either susceptibility or resistance to TB in this study population; (2) genotypes *BB* from *Bsm*I and *TT* from *Taq*I locus occur at a significantly lower frequency in TB patients; (3) genotypes *bb* from *Bsm*I and *tt* from *Taq*I locus occur at a much higher frequency in TB patients.

We did not find a direct association between the VDR *Fok*I polymorphism and the propensity to develop active TB. However, Liu et al.[6] showed that *Fok*I-*ff* homozygotes occurred more frequently in TB patients as compared to the control group in a study conducted in China (OR = 2.18). In agreement with our finding, several other published studies have failed to reveal any effect of *Fok*I on TB.[7,8]

In one of the few studies on *Bsm*I polymorphism and TB, Bornman et al.,[9] in a West African study, demonstrated that the relationship between the two is nonexistent. In contrast, our results revealed a strong association between *bb* genotype and TB irrespective of patient gender (OR = 5.7). Moreover, the *BB* genotype was found to be associated with resistance to PTB in our study (OR = 0.28). Selvaraj et al.[10] also showed genotype *BB* in Indian males to be associated with resistance to PTB.

People with VDR genotype *tt* were underrepresented in the TB group in the study conducted by Bellamy et al.[3] A previous study, however, showed that patients with severe PTB had a trend towards a lower frequency of the *tt* genotype in Gambia. Delgado et al.[11] demonstrated an opposing result, concluding that the *Taq*I genotype has no effect on susceptibility to TB.

doi:10.1016/j.ijid.2009.05.001

Table 1 Genotype frequencies of *Fok*I, *Bsm*I, and *Taq*I polymorphisms of VDR pulmonary TB and control subjects

VDR polymorphism	Genotype frequency			*p*-Values	OR (95% CI)
	TB patients ($N = 60$)		Controls ($N = 62$)		
	Genotype	*n* (%)	*n* (%)		
*Fok*I	*FF*	30 (50)	29 (47)	0.72	1.14 (0.56—2.32)
	Ff	21 (35)	27 (43)	0.34	0.70 (0.35—1.45)
	ff	9 (15)	6 (10)	0.37	1.65 (0.55—4.95)
*Bsm*I	*BB*	13 (22)	31 (50)	0.001	0.28 (0.13—0.61)
	Bb	27 (45)	26 (42)	0.73	1.13 (0.55—2.32)
	bb	20 (33)	5 (8)	< 0.0001	5.7 (1.98—16.45)
*Taq*I	*TT*	8 (13)	33 (53)	< 0.0001	0.135 (0.06—0.33)
	Tt	33 (55)	24 (39)	0.07	1.94 (0.94—3.98)
	tt	19 (32)	5 (8)	0.001	5.3 (1.82—15.30)

VDR, vitamin D receptor; TB, tuberculosis; OR, odds ratio; CI, confidence interval.

Our study has some limitations. First, the sample size is small for a high power genetic susceptibility study. Second, the results could have been strengthened by the inclusion of purified protein derivative results and bacille Calmette—Guérin history in the case and control groups.

In summary, this study found significant differences in the frequency of *Bsm*I and *Taq*I haplogroups of VDR between PTB patients and the healthy control population. Our data support the link between vitamin D receptor alteration and susceptibility to TB. Further research is needed to better characterize this association and to evaluate its clinical significance in TB prevention and control.

Acknowledgement

We appreciate the excellent comments given by Dr. Mary Beth Allen for preparing final version of manuscript.

Conflict of interest: No conflict of interest to declare.

References

1. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* 1997;**349**:1498—504.
2. Comstock GW. Tuberculosis in twins: a re-analysis of the Prophit survey. *Am Rev Respir Dis* 1978;**117**:621—4.
3. Bellamy R, Ruwende C, Corrah T, McAdam KP, Thursz M, Whittle HC, et al. Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene. *J Infect Dis* 1999;**179**:721—4.
4. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. *Gene* 2004;**338**:143—56.
5. Sainz J, Van Tornout JM, Loro ML, Sayre J, Roe TF, Gilsanz V. Vitamin D-receptor gene polymorphisms and bone density in prepubertal American girls of Mexican descent. *N Engl J Med* 1997;**337**:77—82.
6. Liu W, Cao WC, Zhang CY, Tian L, Wu XM, Habbema JD, 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.
7. Wilkinson RJ, Llewelyn M, Toossi Z, Patel P, Pasvol G, Lalvani A, 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.
8. Roth DE, Soto G, Arenas F, Bautista CT, Ortiz J, Rodriguez R, et al. Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. *J Infect Dis* 2004;**190**:920—7.
9. Bornman L, Campbell SJ, Fielding K, Bah B, Sillah J, Gustafson P, 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.
10. Selvaraj P, Narayanan PR, Reetha AM. Association of vitamin D receptor genotypes with the susceptibility to pulmonary tuberculosis in female patients and resistance in female contacts. *Indian J Med Res* 2000;**111**:172—9.
11. Delgado JC, Baena A, Thim S, Goldfeld AE. Ethnic-specific genetic associations with pulmonary tuberculosis. *J Infect Dis* 2002;**186**:1463—8.

Mohammad Mehdi Banoei[a], Mehdi Seyed Mirsaeidi[b*], Massoud Houshmand[a], Payam Tabarsi[c], Golnaz Ebrahimi[c], Laleh Zargari[c], Baharak Houshiar Kashani[a], Mohammad Reza Masjedi[c], Seyed Davood Mansouri[c], Julio Ramirez[b]

[a]*National Institute of Genetic Engineering and Biotechnology, Tehran, Iran*
[b]*Division of Infectious Diseases, Internal Medicine Department, University of Louisville, 627 South Preston Street, Louisville, USA*
[c]*National Research Institute of Tuberculosis and Lung Disease, Shaheed Beheshti Medical Sciences University, Tehran, Iran*

[*]Corresponding author. Tel.: +1 502 852 2852; fax: +1 502 852 1147
E-mail address: m0mirs01@louisville.edu (M. S. Mirsaeidi)

Corresponding Editor: Sheldon Brown, New York, USA