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ORIGINAL ARTICLE

Vitamin D Receptor Gene *Taq*I, *Bsm*I and *Fok*I Polymorphisms in Korean Patients with Tuberculosis

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Background: The active metabolite (1, 25-dihydroxycholecalciferol) of vitamin D (25-hydroxycholecalciferol) leads to activation of macrophages and deficiency of vitamin D seems to be involved in the risk of tuberculosis. The effects of vitamin D are exerted by interaction with the vitamin D receptor (VDR) and may be influenced by polymorphism in the VDR gene. In this study, variation in the VDR gene was investigated in Korean population with tuberculosis. **Methods:** We typed three VDR polymorphisms of restriction endonuclease sites for *Taq*I, *Bsm*I and *Fok*I in 155 patients with tuberculosis and 105 healthy volunteers. **Results:** The frequencies of *Fok*I genotypes determined from TB patients were 29.13% for *FF*, 56.31% for *Ff*, and 14.56% for *ff*. We observed 1.4-fold increased prevalence of the *Ff* genotype in TB patients compared with normal healthy groups ($p=0.0857$). However, there was no significant association between the genotype groups, TB patient and normal control, for *Fok*I polymorphism. There was also no significant association between VDR gene and tuberculosis in another polymorphism (*Bsm*I and *Taq*I). **Conclusion:** Three polymorphisms (*Taq*I, *Bsm*I and *Fok*I) in the VDR gene do not appear to be responsible for host susceptibility to human tuberculosis in Korean population.

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INTRODUCTION

Most of the world's peoples are exposed and infected to a variety of mycobacterial species. Even if rarely pathogenic, mildly virulent mycobacteria such as Bacille Calmette-Guerin (BCG) and most mycobacteria may cause many different of clinical diseases. *Mycobacterium tuberculosis* and *M. leprae*, causing tuberculosis (TB) and leprosy, respectively, are more virulent. Remarkably, only a minority of individuals develops clinical disease, even though infected with virulent mycobacteria. Susceptibility to disease after infection by mycobacteria is influenced by environmental and/or host genetic factors. The interindividual variability of clinical outcome is thought to result, partially, from variability in the genes that control host defense (1,2).

Host genetic factors including major histocompatibility complex (MHC) polymorphisms influence both susceptibility to leprosy and TB (3-5). Non-MHC genes may also play an important role but remain undefined. Genetic variation studies of TB and leprosy have defined a role for HLA-DR (6,7) and variants of the NRAMP1 gene (8) in susceptibility to TB and leprosy.

Vitamin D has an immunoregulatory role mediated by binding to the vitamin D receptor (VDR) in monocyte, macrophages, and lymphocytes (9-11). Vitamin D status seems to be involved in the development of TB (12-15). VDR polymorphisms are occurred in several restriction enzyme sites. FokI and TaqI restriction sites are the best known polymorphisms of VDR gene (13,16). We, herein, investigated VDR genotype on susceptibility to TB and report an analysis of the polymorphisms in the *Fok*I, *Bsm*I, and *Taq*I restriction fragment length polymorphism (RFLP) of the VDR gene (Fig.

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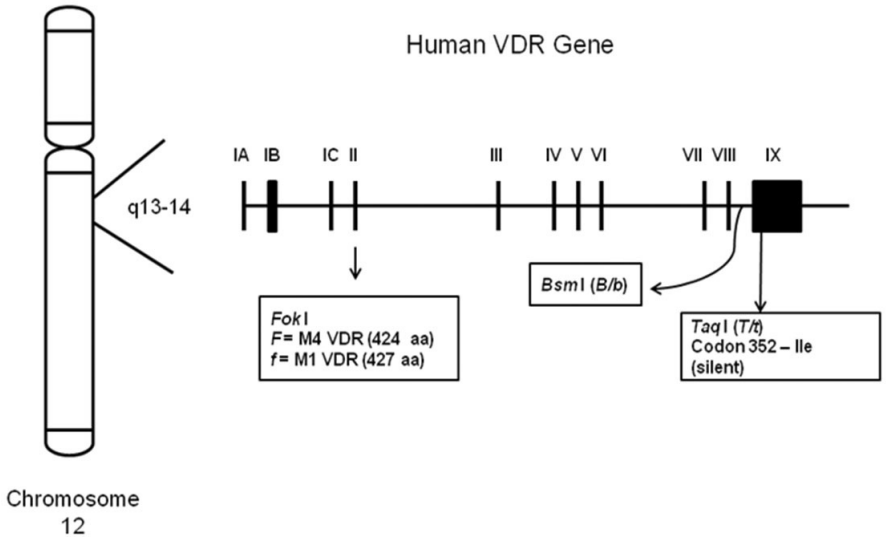

Figure 1. The human vitamin D recepter (VDR) chromosomal genes, containing a total 11 exons, are variably present in VDR transcripts. Three polymorphisms, including a *Fok*I, *Bsm*I, and *Taq*I site, are shown and discussed in the text. The figure is adapted from the schematic view in reference 4.

Table I. Information of subjects for this study

Subjects type	Total no. of subjects	Sex		Average age	Average medication
		Male	Female		
Tuberculosis	155	89	66	40	3.6 (month)
Normal healthy	105	67	38	24	-

1) in TB and normal control samples collected from Korean population.

MATERIALS AND METHODS

Subjects

TB patients were recruited from St. Paul Hospital, Seoul, Korea from 2001 to 2002. *M. tuberculosis* infection was confirmed by AFB (Acid-Fast Bacilli) staining in their sputum and by culture. The control group comprised healthy, blood donors with no history of TB or other immune diseases.

The TB patients comprised 89 males and 66 females, and their ages ranged from 17 to 69 years. All patients were diagnosed with pulmonary TB and their mean duration of therapy was about 6.6 years. Healthy normal control subjects with no history of previous tuberculosis consisted of 67 males and 38 females, with age ranging from 21 to 52 years (Table I). Written consent was obtained from each participant.

Preparation of genomic DNA

The genomic DNA from the study subjects was isolated from PBMC. We prepared PBMCs from heparinized blood, isolated by centrifugation through Ficoll-Hypaque density gradient, then washed with DPBS (Sigma Chemical Co., St. Louis, MO. USA). 10^6 of PBMC were treated with PCR-K buffer [10× PCR buffer 1 ml, NP-40 40 μl, Tween-20 45 μl, protease K (20 mg ml^{-1}) 30 μl, D.W. 8.8 ml] 1 ml, incubated at 58°C for 1 hr and then incubated at 95°C for 10 min to inactivate proteinase K. This product was used as a template DNA for PCR.

VDR polymorphisms

DNA was used in the PCR amplification of sequences containing previously described VDR restriction-fragment-length polymorphisms defined by the restriction endonucleases *Taq*I, *Fok*I, and *Bsm*I. The primer sequences used in this study were as follows: 5'-cag agc atg gac agg gag caa g-3' and 5'-ggt ggc ggc agc gga tgt acg t-3' for *Taq*I; 5'-agc tgg ccc tgg cac tga ctc tgc tct-3' and 5'-atg gaa aca cct tgc ttc ttc tcc ctc-3' for *Fok*I; 5'-aac ttg cat gag gag gag cat gtc-3' and 5'-gga gag gag cct ctg tcc cat ttg-3' for *Bsm*I.

The cycling profile involved denaturation at 94°C for 15 sec, annealing at 65°C (*Taq*I) and 55°C (*Fok*I and *Bsm*I) for 30 sec, and extension at 72°C for 30 sec for 35 cycles. Final extension was continued at 72°C for 10 min. The amplification was carried out in a GeneAmp PCR system 9600 (Applied Biosystems, Branchburg, CT, USA).

PCR products were digested overnight with restriction endo-

nuclease so the reaction could proceed to completion, in accordance with the manufacturer's instructions (Roche Molecular Biochemicals, Indianapolis, IN, USA). Digested products were analyzed by electrophoresis in a 2% agarose gel and ethidium bromide staining.

Statistical analysis

The genotype frequencies of each of the SNPs were compared by the chi-square tests, Fisher's exact test and Cochran-Armitage Trend Test. Logistical regression analyses with three alternative models (additive, dominant and recessive) were used to calculate the odds ratios (OR) and 95% confidence intervals (CI) of each SNP. Data analyses were performed using the computer software SAS 9.1.3 (SAS Inc., Cary, NC, USA). All tests were two tailed and $p<.05$ was considered statistically significant.

Table II. Vitamine D receptor (VDR) gene polymorphisms in patients with tuberculosis and healthy controls

Genotype	TB patients (%)	Healthy controls (%)
TaqI		
TT	134 (89.93)	85 (90.43)
Tt	14 (9.40)	8 (8.51)
Tt	1 (0.67)	1 (1.06)
Total	149 (100)	94 (100)
Allele frequencies		
T	282 (94.63)	178 (94.68)
t	16 (5.37)	10 (5.32)
BsmI		
BB	2 (1.33)	0 (0)
Bb	13 (8.67)	8 (9.64)
bb	135 (90.0)	75 (90.36)
Total	150 (100)	83 (100)
Allele frequencies		
B	17 (5.67)	8 (4.82)
b	283 (94.33)	158 (95.18)

Table III. Distribution of *Fok*I genotypes in TB patients and normal subjects

Genotype	Subjects		p value
	Normal healthys (%)	TB patients (%)	
FF	41 (39.05)	30 (29.13)	
Ff	43 (40.95)	58 (56.31)	0.0857
ff	21 (20.00)	15 (14.56)	
Total	105 (100)	103 (100)	
Allele frequencies			
F	105 (55.26)	118 (57.28)	0.6858
f	85 (44.74)	88 (43.72)	
Phenotype frequencies			
F positive	84 (56.76)	88 (54.66)	0.7107
f positive	64 (43.24)	73 (45.34)	

RESULTS

The results of genotyping of VDR gene for TB patients and healthy control are summarized in Table 2 and 3. First, for the *Taq*I and *Bsm*I polymorphism, the TB patient and normal control groups had similar distribution, and there was no significant difference in allele frequency distribution between patients and controls. In Table II, genotype frequency analysis of *Taq*I site in TB patients showed that the largest group consisted of *TT* homozygotes (89.93% of 149 genotypes), followed by the *Tt* heterozygous group (9.4%), while *tt* homozygotes were 0.67% (1 of 149 genotypes). In contrast, while *bb* homozygotes was the largest group (90.0% of 150 genotypes) in *Bsm*I genotype, *Bb* heterozygote and *bb* homozygote are 8.67% and 1.33%, respectively.

PCR products restricted with *Fok*I were showed in Fig. 2. Three bands after treatment with enzyme were showed as *FF* homozygotes (266 bp), *Ff* heterozygotes (193 bp), and *ff* homozygotes (73 bp) according to the restriction pattern (Fig. 2). Table III showed the *Fok*I genotypes determinants for TB patients and normal groups. The frequencies of the *Fok*I genotypes were 29.13% for *FF*, 56.31% for *Ff*, and 14.56% for *ff*. We observed 1.4-fold increased prevalence of the Ff genotype in TB patients compared with normal healthy groups ($p=0.0857$). However, there was no significant association between the genotype groups, TB patient and normal control, for *Fok*I polymorphism (Table III).

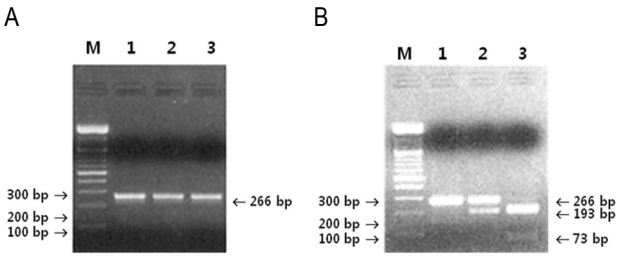

Figure 2. *Fok*I restriction patterns of the various genotypes. PCR products before (A) and after (B) treatment of enzyme were showed. B. Three restriction fragments were present at 266, 193, and 73 bp. The *FF* genotype (B-1) had only the 266 bp band, the *ff* genotype (B-3) had the two bands at 193 and 73 bp, and the *Ff* heterozygous genotype (B-2) had the three bands.

Table IV. The differences of genotype frequencies of *Fok*I and *Taq*I VDR gene between patients from Korea and the other countries

*Fok*I	*FF* (%)	*Ff* (%)	*ff* (%)	Total (%)	Reference
Korean	30 (29.13)	58 (56.31)	15 (14.56)	103 (100)	In this study
European	40 (49.66)	52 (46.55)	13 (8.79)	105 (100)	19
African	56 (46.34)	38 (43.45)	4 (8.21)	98 (100)	19
Gujarati Asians*	52 (57)	31 (34)	8 (9)	91 (100)	13
*Taq*I	*TT* (%)	*Tt* (%)	*tt* (%)	Total (%)	Reference
Korean	134 (89.93)	14 (9.4)	1 (0.67)	149 (100)	In this study
Cambodia	325 (90.8)	30 (8.4)	3 (0.8)	358 (100)	20
Gambia	204 (50)	177 (43)	27 (6.6)	408 (100)	17
Gujarati Asians*	39 (43)	46 (51)	6 (6)	91 (100)	13

*Gujarati Asians were Hindu, resident in London (living in Harrow, UK).

DISCUSSION

In human, mycobacterial pathogenicity varies from one mycobacterial species to another. *M. tuberculosis* and related species of TB complex are the agents of human TB, the leading infectious disease world-wide.

There is much variability among individuals in the response to mycobacterial infections, but it is not known why certain people develop disease when challenged with mycobacteria and others remain healthy. However, the intrinsic virulence of each mycobacterial species is not the sole pathogenic factor as the outcome of mycobacterial infection depends on the genetic backgrounds of the infected individual. The molecular basis of the genetic vulnerability underlying most mycobacterial diseases in human remains is, in large, unknown.

Recently genetic variation has been shown to be associated with single nucleotide polymorphisms (SNP) in the vitamin D receptor (VDR) gene in many populations of leprosy and TB (13,17,18). Epidemiological evidence suggests that there is a link between vitamin D deficiency and susceptibility to leprosy and TB.

Vitamin D metabolism leads to activation of macrophages and restricts the intracellular growth of mycobacteria. This effect of vitamin D may be influenced by polymorphisms at three sites (*Taq*I, *Bsm*I and *Fok*I) in the vitamin D receptor (VDR) gene (11). Recent studies also have implicated variation of the vitamin D receptor (VDR) gene in susceptibility to several diseases, including hepatitis and tuberculosis (17).

Therefore, we studied the association between VDR polymorphism and TB in Korean population with pulmonary TB and we found that there is no significant association in our analysis between TB patients and VDR polymorphisms. However, we found the differences of genotype frequencies of *Taq*I and *Fok*I VDR between patients from Korean and the other countries including European, African, and Gujarati Asian previously reported (Table IV). For example, while the relative genotype frequencies of *Fok*I polymorphism in Korean patients were *FF* 29.13, *Ff* 56.31, and *ff* 14.56%, the genotype frequencies in African were *FF* 46.34, *Ff* 43.45, and *ff* 8.21%. And the relative genotype frequencies of *Taq*I polymorphism in Korean were *TT* 89.93, *Tt* 9.4, and *tt* 0.67%, whereas the frequencies determined on Gambia (African) were *TT* 50.0, *Tt* 43.0, and *tt* 6.6%. However it is uncertain if there are racial differences involved in environmental or genetic factors.

Although several investigators reported and suggested that the VDR polymorphism may be of immunoregulatory importance for many disease processes, it is not clear that the polymorphism determine susceptibility to the development of clinical disease or susceptibility to infection.

Further studies will be required to investigate how VDR polymorphism may influence susceptibility to infectious disease or development of clinical disease.

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CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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