Vitamin D Receptor Gene TaqI, BsmI and FokI 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 TaqI, BsmI and FokI in 155 patients with tuberculosis and 105 healthy volunteers.

Results: The frequencies of FokI 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 FokI polymorphism. There was also no significant association between VDR gene and tuberculosis in another polymorphism (BsmI and TaqI).

Conclusion: Three polymorphisms (TaqI, BsmI and FokI) in the VDR gene do not appear to be responsible for host susceptibility to human tuberculosis in Korean population.

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 FokI, BsmI, and TaqI restriction fragment length polymorphism (RFLP) of the VDR gene (Fig.
Table I. Information of subjects for this study

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

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 TaqI, FokI, and BsmI. The primer sequences used in this study were as follows: 5’-cag aac ctt tcc ttg ctc ctc ctc tct-3’ for *TaqI*; 5’-gcc gcc gggagg gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgcc gccgc
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.

**RESULTS**

The results of genotyping of VDR gene for TB patients and healthy control are summarized in Table 2 and 3. First, for the *TaqI* and *BsmI* 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 *TaqI* 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 *BsmI* genotype, Bb heterozygote and bb homozygote are 8.67% and 1.33%, respectively.

PCR products restricted with *FokI* 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 *FokI* genotypes determinants for TB patients and normal groups. The frequencies of the *FokI* 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 *FokI* polymorphism (Table III).

### Table II. Vitamin 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 *FokI* genotypes in TB patients and normal subjects

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

**Figure 2.** *FokI* 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 FokI and TaqI VDR gene between patients from Korea and the other countries

|        | FokI         |        | TaqI         |        |        | Reference |
|--------|--------------|--------|--------------|--------|--------|-----------|
|        | FF (%)       | Ff (%) | ff (%)       | Total (%) |        |           |
| 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 |

|        | TT (%)       | Tt (%) | tt (%)       | Total (%) | Reference |
|--------|--------------|--------|--------------|-----------|-----------|
| Korean | 134 (89.93)  | 14 (9.4) | 1 (0.67)     | 149 (100) | In this study |
| Cambod | 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 (TaqI, BsmI and FokI) 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 TaqI and FokI 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 FokI 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%, respectively. And the relative genotype frequencies of TaqI polymorphism in Korean were TT 89.93, Tt 9.40, and tt 0.67%, whereas the frequencies determined on Gambia (African) were TT 50.00, Tt 43.00, and tt 6.60%. 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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