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ORIGINAL ARTICLE

VDR and *VDBP* genes polymorphisms associated with susceptibility to tuberculosis in a Han Taiwanese population

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Abstract *Background:* The active metabolite (1, 25-dihydroxycholecalciferol) of vitamin D (25-hydroxycholecalciferol) leads to the activation of macrophages and the deficiency of vitamin D seems to be involved in the risk of tuberculosis (TB). The effects of vitamin D are exerted by interaction with the vitamin D receptor (VDR) and vitamin D receptor binding protein (VDBP) may be influenced by polymorphisms in the *VDR* and *VDBP* genes. In this study, variation in the *VDR* and *VDBP* genes was investigated in a Taiwanese population with TB.
Methods: We typed four *VDR* polymorphisms of restriction endonuclease sites for *ApaI*, *TaqI*, *BsmI*, and *FokI* and three *VDBP* polymorphisms—Thr420Lys, Asp416Glu, and Cys299Cys—in 198 patients with TB and 170 healthy volunteers.
Results: VDR TaqI, *VDR BsmI*, and *VDBP Asp416Glu* were significantly associated with TB susceptibility. Odd ratios of risk genotypes of the above three polymorphisms were 2.16 (95% confidence interval 1.01, 4.65), 2.14 (95% confidence interval 1.06, 4.31), and 2.24 (95% confidence interval 1.04, 4.80), respectively. VDBP haplotype analysis showed Gc1f carriers associated to TB.
Conclusion: The polymorphisms in the *VDR* and *VDBP* genes appeared to be responsible for host susceptibility to human TB in a Taiwanese population.

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Introduction

According to the World Health Organization (WHO), approximately 8.6 million cases (8.3—9.0 million) of tuberculosis (TB) were estimated to have occurred in 2012, of which approximately 2.9 million were female. Most cases are estimated to have occurred in Asia and Africa (58% and 27%, respectively), with the highest incidence in India (2.0—2.4 million) and China (0.9—1.1 million), together accounting for 38% of the total number of cases.[1] Previous studies in Taiwan have demonstrated a five-fold higher incidence of TB among aborigines as compared to Han Chinese.[2] In addition, polymorphisms of the *NRAMP1* gene appear to be associated with susceptibility to TB among aborigines, but not among the Han Chinese population.[2] Our previous study showed polymorphisms in the interferon-γ gene to be associated with TB in the Han Taiwanese population.[3] The genetic susceptibility to TB in Han Chinese living in Taiwan is remains unknown.

Calcitriol, (also known as 1, 25-dihydroxyvitamin D_3 and 1, 25$(OH)_2D_3$), an immunomodulatory hormone, acts via the vitamin D receptor (VDR) to alter genomic signaling.[4] It regulates the differentiation and growth of various immune cells and the derivatives of calcitriol have been shown to inhibit the functional differentiation of dendritic cells, cytotoxic T-cells, and helper T-cells.[5,6] Calcitriol inhibits the Th1 cytokine production and augments production of T-cell suppressing cytokines, TGF-β1 and IL-4.[7] Studies have shown that calcitriol inhibits NK cell activity and differentiation of lymphokine-activated killer cells.[8] It suppresses the growth of *Mycobacterium tuberculosis* in mononuclear phagocytes[9] and toll-like receptor activation of human macrophages has been shown to upregulate expression of VDR and vitamin D 1α-hydroxylase genes leading to induction of cathelicidin and killing of *M. tuberculosis*.[10,11] *VDR* gene variants have a wide role in innate immunity and specifically in TB and are associated with TB.[12—16] The most well-known polymorphisms in the 3′ untranslated region (UTR) and start codon of *VDR* gene are *ApaI*, *BsmI*, *TaqI*, and *FokI*, respectively. Some studies have indicated a strong association between *BsmI* gene polymorphism and pulmonary TB.[12,17] It was seen that the f/f genotype for *FokI* and t/t genotype for *TaqI* occurred more frequently in TB patients.[15,18]

Vitamin D metabolites in the circulation are bound to vitamin D binding protein (DBP), a widely expressed multifunctional 58 kDa serum glycoprotein encoded on chromosome 4. The *DBP* locus is among the most polymorphic known.[19] Two common polymorphisms at codons 416 (GAT→GAG, Asp→Glu) and 420 (ACG→AAG, Thr→Lys) of exon 11 of the *DBP* gene (defined by the presence of restriction endonuclease sites for *HaeIII* and *StyI*, respectively) give rise to the three major electrophoretic variants of DBP, termed group-specific component 1 fast (Gc1F), Gc1 slow (Gc1S), and Gc2. These variants differ in their functional characteristics: the Gc1F and Gc1S variants have been reported to have a greater affinity for 25 (OH)D than the Gc2 variant,[20] potentially leading to more efficient delivery of 25(OH)D to the target tissues, while the Gc2 variant is associated with decreased circulating concentrations of 25(OH)D, 1,25(OH)2D and DBP.[21,22] The Gc2/2 genotype of *VDRP* was strongly associated with susceptibility to active tuberculosis in Gujarati Asians, compared with Gc1/1 genotype.[23]

In this study, we examined the polymorphisms of *VDR* and *VDBP* to gain a better understanding about the possible correlation between genetic risk factors and susceptibility to TB in Taiwanese patients.

Materials and methods

Participants

A total of 198 patients who were treated for active TB at the General Taoyuan Hospital (Taoyuan, Taiwan) between 2009 and 2011 were surveyed consecutively. The inclusion criteria were: adult patients newly diagnosed with active TB, having evident lesions of TB by simple X-ray, computed tomography, and positive results of sputum smears and cultures for mycobacteria. In the control group, 170 volunteers without active TB or a history of TB were enrolled. Individuals with latent TB (confirmed by physician and Quantiferon test) history were excluded.

Written informed consent was obtained from each patient and volunteer enrolled in this study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Taoyuan General Hospital.

DNA preparation

Genomic DNA was extracted from oral swabs collected from the 198 TB patients and 170 non-TB volunteers using a QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. The extracted genomic DNA was analyzed using agarose gel electrophoresis and quantitatively determined by spectrophotometry, and stored at −80°C until use.

Single nucleotide polymorphism genotyping

All single nucleotide polymorphism (SNP) genotyping was performed using TaqMan SNP genotyping assays (ABI: Applied Biosystems Inc., Foster City, CA, USA). The primers and probes of the selected SNPs were from an ABI assay on demand kit. Reactions were carried out according to the manufacturer's protocol (TaqMan SNP Genotyping Assays, protocol, Part Number 4332856 Rev. C). The probe fluorescence signal detection was performed using an ABI Prism 7900 Real-Time PCR System.

Statistical analysis

The quality of the genotype data were evaluated by Hardy—Weinberg equilibrium proportion tests. The association analyses were tested by the χ^2 test. Odds ratios and 95% confidence intervals were calculated from contingency tables. SNP(s) showing significant association ($p \leq 0.05$) in the tests were further evaluated using logistic regressions adjusted for age and sex in odds ratio analysis. All

statistical analyses were performed with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

Results

Demographic information of the study participants

In this study, 198 patients diagnosed with TB and 170 control individuals without history of TB infection were enrolled. There were significant differences in sex and age between groups. Male sex was more prevalent in the case group (Table 1). The average ages of the case and control group were found to have no significant differences (Table 1).

VDR and VDBP variant(s) associated with susceptibility to tuberculosis

Seven selected SNPs were genotyped by TaqMan SNP genotyping assays. None of the genotype distributions of the tested SNPs in the study participants deviated from the Hardy–Weinberg equilibrium. The strengths of associations and genotype frequencies of all selected SNPs with TB are summarized in Table 2. None of the SNPs appeared to be significantly associated with TB.

The odds ratio analysis adjusted by sex and age showed that AA of rs731236, GG of rs1544410 and A carriers (AA+AC) of rs7041 were risk genotypes for susceptibility to TB (Table 3).

The polymorphisms rs4588 and rs7041 of *VDBP* were further analyzed according Gc2/Gc1 haplotype. The Gc1f carriers were associated with susceptibility of TB (Table 4).

Discussion

In this study, we revealed the SNPs rs731236 (*TaqI*) and rs1544410 *(BsmI*) of *VDR* and rs7041 (Asp416Glu) of *VDBP* associated with susceptibility of TB in the Han Taiwanese population. Genotypes AA of rs731236, GG of rs1544410 and A carriers (AA+AC) of rs7041 were risk genotypes for susceptibility to TB.

The *ApaI* and *BsmI* polymorphisms were located in the intron region between exon 8 and exon 9 of the VDR gene. Although the nucleotide changes of the *ApaI* and *BsmI* polymorphisms generated no changes in the amino acid or the structure of the expressed VDR protein, they might be in linkage disequilibrium with other functional polymorphisms which regulated VDR gene expression. Earlier studies have provided evidence of differential luciferase activity for the two 3′ UTR variants that were linked to the most frequent haplotypes.[24] A previous meta-analysis showed recessive model (aa) of the ApaI polymorphism and dominant model (Bb+bb) for the *BsmI* polymorphism both appeared to have a protective role on tuberculosis development in the European population.[25] Our result showed homozygous of major allele of rs1544410 (*BsmI*: BB) of *VDR* is a risk genotype for TB (Table 2), which is consistent with the aforementioned meta-analysis in the European population.[25] The *ApaI* polymorphism did not show association to TB in our study population.

The mRNA coded from the *TaqI* t allele of the VDR gene would be more stable than the mRNA from the T allele of the *VDR* gene.[26] Our result showed that rs731236 major

Table 1 Demographic data of the study participants.

	TB (198 cases)	Non-TB (170 controls)	*p*
Age (y), mean ± SD	55.78 ± 10.99	55.76 ± 18.62	0.199
Sex (*n*)	Male: 137 Female: 61	Male: 79 Female: 91	< 0.001

Age was tested by *t* test; sex was tested by χ^2 test.
SD = standard deviation TB = tuberculosis.

Table 2 Genotyping frequencies of selected single nucleotide polymorphisms (SNPs) of VDR and VDBP in the tuberculosis (TB) and non-TB groups and results of the χ^2 test.

Polymorphism	SNP ID	allele	Non-TB	TB	*p*
		1/2*	11/12/22**	11/12/22**	
VDR					
ApaI	rs7975232	A/C	16/65/89	17/78/103	0.950
TaqI	rs731236	A/G	149/20/1	186/12/0	0.083
BsmI	rs1544410	A/G	0/24/146	1/14/183	0.058
FokI	rs2228570	A/G	32/87/51	50/104/44	0.144
VDBP					
Thr420Lys	rs4588	A/C	15/62/93	14/80/104	0.669
Asp416Glu	rs7041	A/C	80/69/21	102/84/12	0.106
Cys299Cys	rs4725	C/T	1/22/147	4/24/170	0.488

*1: allele 1, 2: allele 2; **11: homozygous of allele 1, 12: heterozygous, 22: homozygous of allele 2.

Table 3 Odds ratio analysis of selected single nucleotide polymorphisms (SNPs).

SNP ID	Genotype	Non-TB	TB	*p*	OR (95% CI)*
rs7975232	AA+AC	81	89	0.949	n.d.
(ApaI)	CC	89	103		
rs731236	AA	149	186	0.035	2.16
(TaqI)	AG+GG	21	12		(1.01, 4.65)
	(ref.)				$p = 0.048$
rs1544410	AA+AG	24	15	0.042	2.14
(BsmI)	(ref.)	146	183		(1.06, 4.31)
	GG				$p = 0.034$
rs2228570	AA+AG	119	154	0.089	n.d.
(FokI)	GG	51	44		
rs4588	AA+AC	77	94	0.676	n.d.
(Thr420Lys)	CC	93	104		
rs7041	AA+AC	149	186	0.035	2.24
(Asp416Glu)	CC (ref.)	21	12		(1.04, 4.80)
					$p = 0.039$
rs4725	CC+CT	23	28	0.865	n.d.
(Cys299Cys)	TT	147	170		

* Adjusted for sex and age by logistic regression.
CI = confidence interval; n.d. = not determined; OR = odds ratio; ref. = reference genotype.

Table 4 VDRP polymorphisms rs4588/rs7041 genotype combinations and association to tuberculosis (TB).

rs4588/rs7041	AA/AA	AC/AC*	AC/AA	CC/AA	CC/AC	CC/CC
	Gc2/Gc2	Gc2/Gc1s	Gc2/Gc1f	Gc1f/Gc1f	Gc1f/Gc1s	Gc1s/Gc1s
Non-TB	15	29	33	32	40	21
TB	14	30	50	38	54	12
	Non-Gc1f carriers**		Gc1f carriers**			
Non-TB	65		105		$\chi^2 = 4.11$	
TB	56		142		$p = 0.0426$	
	OR = 1.57(95% CI: 1.014, 2.431)					

* Frequency of the rs4588/rs7041 AC haplotype is extremely low due to linkage disequilibrium between loci; therefore individuals heterozygous at both loci were assumed to carry AA/CC haplotypes: **: non-Gc1f carriers included Gc2/Gc2, Gc2/Gc1s and Gc1s/Gc1s, Gc1f carriers included Gc2/Gc1f, Gc1f/Gc1f and Gc1f/Gc1s.
CI = confidence interval; OR = odds ratio.

allele homozygous (as *TaqI*, TT) is a risk genotype for TB (Table 2). A previous study revealed that the *TaqI* SNP in exon 9 near the 3′ UTR was in linkage disequilibrium with the *ApaI* and *BsmI* polymorphisms,[27] which may explain the same protective effect by the two variant alleles of the *TaqI* and *BsmI* polymorphisms on tuberculosis.

The *FokI* SNP was located within the exon 2 of the *VDR* gene, and evidence of *FokI* functionality has already been obtained.[28] Thus, the f allele of *FokI* might decrease the activity of the VDR protein and deter the binding of active vitamin D and VDR. Three meta-analyses on the *FokI* polymorphism were in accordance and indicated that the f allele in a recessive model would increase the risk of tuberculosis, and the same effect was found in the Chinese population but not for other ethnicities.[14,25,29] The association between *FokI* and TB in our study population did not show statistical significance, the marginal significant (Table 2; OR analysis, $p = 0.089$), which may due to the small sample size. The analysis of the effect of the *FokI* polymorphism on tuberculosis was underpowered.

In 1987 a possible correlation was proposed between the homozygous Gc1F-phenotype and susceptibility to HIV infection/severity of HIV-related disease. Gc2-2 by contrast, should perform a protective role.[30] Several later studies refuted this statement.[31–33] Gc2 homozygotes were more common in TB relative to controls in two studies in Asian populations,[23,34] but the risk appears to depend on an interaction between vitamin D status and genotype.[36] In Caucasian Russians, no GC genotype was associated with TB.[35] The association might be consistent with their reduced ability to convert GC to macrophage-activating factor, but requires further study as to the reasons for synergy with vitamin levels. Our study was inconsistent with previous studies and showed an association between Gc1F carriers and risk of TB.

Previously, only one study had evaluated the DBP phenotype in TB patients and no differences were seen among patients and the control group.[34] In that study, a 33% frequency of Gc2 in TB patients was slightly but not significantly higher than in the control group (26%), and this elevation was at the expense of both Gc1F and Gc1S alleles.[34] Further studies are necessary to understand the physiological role of VDBP and its phenotypes on susceptibility to TB and other diseases.

If the vitamin D axis plays a role in TB pathogenesis,[36] variation within genes such as *GC* and *VDR* could be relevant in promoting resistance or susceptibility to the infection. VDR has been more widely studied and genetic variation within it appears to influence lymphocyte response to *M. tuberculosis*.[37] Nevertheless a meta-analysis of *VDR* studies was inconclusive,[38] perhaps due to small study sizes and population heterogeneity. HIV status in particular may influence the apparent effect of susceptibility loci, perhaps because in HIV-positive individuals this surpasses the small risk attributable to genetic factors.[39] Therefore, further investigation, including functional and genetic studies, for *VDR* and *VDBP* involving the pathology of TB are necessary.

Conflicts of interest

All authors have no conflicts of interest to declare.

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