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ORIGINAL RESEARCH

Association of Single-Nucleotide Polymorphisms in the *VDR* Gene with Tuberculosis and Infection of Beijing Genotype *Mycobacterium tuberculosis*

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Background: The aim of the present study was to investigate the association between vitamin D receptor (*VDR*) gene polymorphism and tuberculosis susceptibility, as well as the potential interaction of host genetic factors with the heterogeneity of *Mycobacterium tuberculosis* in the population from Xinjiang, China.

Methods: From January 2019 to January 2020, we enrolled 221 tuberculosis patients as the case group and 363 staff with no clinical symptoms as the control group from four designated tuberculosis hospitals in southern Xinjiang, China. The polymorphisms of Fok I, Taq I, Apa I, Bsm I, rs3847987 and rs739837 in the *VDR* were detected by sequencing. *M. tuberculosis* isolates were collected from the case group and identified as Beijing or non-Beijing lineage by multiplex PCR. Propensity score (PS), univariate analysis and multivariable logistic regression models were used to perform the analysis.

Results: Our results showed that the allele and genotype frequencies of Fok I, Taq I, Apa I, Bsm I, rs3847987 and rs739837 in *VDR* were not correlated with tuberculosis susceptibility or lineages of *M. tuberculosis*. Two out of six loci of the *VDR* gene formed one haplotype block, and none of the haplotypes was found to correlate with tuberculosis susceptibility or lineages of *M. tuberculosis* infected.

Conclusion: Polymorphisms in the *VDR* gene may not indicate susceptibility to tuberculosis. There was also no evidence on the interaction between the *VDR* gene of host and the lineages of *M. tuberculosis* in the population from Xinjiang, China. Further studies are nonetheless required to prove our conclusions.

Keywords: vitamin D receptor, *VDR*, polymorphism, tuberculosis, *Mycobacterium tuberculosis*, Beijing lineage

Introduction

Prior to the new coronavirus (COVID-19) pandemic, tuberculosis was the leading cause of death from a single infectious disease. About 10.6 million new cases of tuberculosis, with an incidence rate of 134 per 100,000, of which 6.6% were co-infected with human immunodeficiency virus (HIV), and an estimated 1.6 million deaths from tuberculosis were reported globally in 2021.[1] Tuberculosis is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* complex, which is spread mainly from person to person through the respiratory pathway. At present, about a quarter of the world's population is infected with *M. tuberculosis*. For these people, the lifetime risk of developing tuberculosis is about 5–10%,[1] which reflects the genetic differences in human susceptibility to active and latent tuberculosis.[2]

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It has been reported that host and pathogen have evolved mechanisms and relationships that greatly influence the outcome of infection,[3] and innate immunity-related genes of the host genome provide a good model for studying the selection pressure exerted by microorganisms.[4] For example, the vitamin D (1,25-dihydroxyvitamin D3) nuclear receptor (vitamin D receptor, VDR), a trans-acting transcription factor, mediates the innate immune response by enhancing the expression of several antimicrobial peptides and participates in the elimination process of *M. tuberculosis*.[5] By interacting with VDR, vitamin D could promote phagolysosome fusion and autophagy while inducing anti-inflammatory cytokine secretion that prevents excessive lung pathology and then counteracts multiple virulence mechanisms used by *M. tuberculosis* to evade the host immune response and establish infection.[3]

The *VDR* gene is located on chromosome 12q13.11, and its Mendelian Inheritance Database (OMIM) number is 601769, including multiple single-nucleotide polymorphism sites such as FokI, TaqI, ApaI and BsmI. Previous literature shows that *VDR* gene polymorphisms can affect the host's susceptibility to tuberculosis and the pharmacokinetic levels of first-line antitubercular drugs,[5–7] but the results did not reach a consensus in distinct ethnic groups. Sha et al[8] found that Bsm I-Bb and bb genotypes and Fok I-Ff and ff genotypes in *VDR* gene increasing the risk of tuberculosis susceptibility in adolescents. Zhang et al[9–11] found that the allele f and the genotype ff in the Fok I were respectively associated with tuberculosis susceptibility in the Kazakh and in the Han population; no correlation was observed between the Taq I locus polymorphism and tuberculosis susceptibility in the Kazakh, Uyghur and Han populations in Xinjiang. The above studies classified the allele genes and genotypes according to PCR amplification product digestion – gel electrophoresis imaging, which provided clues on the associations between *VDR* polymorphism and tuberculosis susceptibilities. Xu[12] conducted a meta-analysis of 42 studies and found that the variation of the *VDR* gene BsmI and TaqI sites was significantly associated with tuberculosis, and further subgroup analysis by race showed that both BsmI and TaqI site variations were associated with tuberculosis in South Asians, inconsistent with Wang's[13] meta-analysis of 54 studies. We speculated that only investigating the polymorphic variants of human genes without considering the factors from pathogens made above researches less repeatable.

Previous studies showed that the Beijing lineage of *M. tuberculosis* is the most prevalent in the national wide of China.[14,15] However, lineage distributions of *M. tuberculosis* and ethnic distributions of tuberculosis patients have been reported to be variable in different geographic locations in China.[15–17] For example, the ratio of Beijing family strains in northern provinces is much higher than that in southern provinces.[15] Compared with the neighboring provinces, such as Tibet, Gansu and Inner Mongolia, the proportion of Beijing family in Xinjiang is obviously lower.[16,17] It is unclear whether *VDR* gene polymorphisms affect the geographical transmission of *M. tuberculosis* with different lineages, and it would be helpful to clarify the susceptibility mechanism to tuberculosis in Xinjiang, China, by combining the lineage of *M. tuberculosis* with the polymorphic variants of the human *VDR* gene. The purpose of this study was to evaluate six single-nucleotide polymorphisms (SNPs) Fok I (rs2228570), Taq I (rs731236), Apa I (rs7975232), Bsm I (rs1544410), rs3847987 and rs739837 in the VDR and their potential interaction with *M. tuberculosis* lineage in the population from Xinjiang.

Methods

Ethical Approval

This study was approved by the Ethics Committee of the Eighth Affiliated Hospital of Xinjiang Medical University (XJMU8HEC-20161215). The patients with tuberculosis and healthy staff in the designated hospitals were included in the present study only after we obtained written informed consent from themselves, or from their parents/guardians in cases where the patient was a child (≤18 years of age).

Estimation of Sample Size

Sample size was estimated based on a minimum allele frequency (MAF) of 20% for the smaller Taq I locus with an expected OR of 2.5, α=0.05 (bilateral) and β=0.20 for an unpaired case–control study. The sample size required for the case and control groups was estimated to be at least 126 individuals per group, calculated using PASS 15.0 software (NSCC, USA).

Participants

From January 2019 to January 2020, the tuberculosis patients were enrolled consecutively in four designated tuberculosis hospitals in Xinjiang (ie, the Eighth Affiliated Hospital of Xinjiang Medical University, Kashgar Pulmonary Hospital, Kuqa County Infectious Disease Hospital and Wushi County People's Hospital) and their basic information were recorded. All cases were in compliance with the tuberculosis diagnosis criteria issued by the National Health and Family Planning Commission of the People's Republic of China.[18,19] Patients with HIV-positive or severe primary diseases were not included in this study.

The control group consisted of the staff in these hospitals with no clinical symptoms. They were confirmed to have negative TSPOT.TB results.

Specimen Collection and DNA Extraction

After all participants signed the informed consent forms and joined the study, 5 ml of venous blood from each participant were collected in EDTA anticoagulant tubes. The nucleic acid extraction kit (magnetic bead method) and AU1001-96 automatic nucleic acid extractor provided by Bio teke Biotechnology Corporation, Wuxi, China, were then used to extract DNA from the blood samples. A total of 65 clinical isolates of M. tuberculosis from the case group were collected, and their genomic DNA was extracted through the cetyltrimethylammonium bromide (CTAB) method.[20] All extracted DNA was quantitated by spectrophotometry using the NanoDrop One (Thermo Scientific, Massachusetts, USA) and stored at −20°C until analyzed.

Single-Nucleotide Polymorphism of *VDR* Gene

The six polymorphic SNPs Fok I, Taq I, Apa I, Bsm I, rs3847987 and rs739837 in *VDR* gene were studied to find out whether the polymorphisms are associated with tuberculosis susceptibility, and their specific information can be seen at Table 1. The primers were synthesized by Beijing Tianyi Huiyuan Biotechnology Co., Ltd, Beijing, China, and the sequences were as follows: 5'-ATCATGTATGAGGGCTCCGAAG-3' and 5'-GCCGCATGTTCCATGGACATTG-3' for Fok I, 5'-AAGGG GCGTTAGCTTCATGC-3' and 5'-TCACCGGTCAGCAGTCATAGA-3' for Taq I and Apa I, 5'-CGGAAGAGGTC AAGGGTCAC-3' and 5'-CCTGAAGGGAGACGTAGCAA-3' for Bsm I, 5'-GTGCTCCAGTGATGGGAAGA-3' and 5'- GCTTTTCCCCGGTCCCTTGA-3' for rs3847987 and rs739837. The total volume of the PCR was 25 μL, including 12.5 μL PCR mixture, 1 μL DNA, 10 ng upstream primer, 10 ng downstream primer and 9.5 μL ddH_2O. PCR conditions were 95°C for 3 min, followed by 35 cycles of 95°C for 30s, 62.1°C (Fok I) or 61.3°C (Taq I and Apa I) or 62.5°C (Bsm I) or 61.8°C (rs3847987 and rs739837) for 30s, and 72°C for 30s, and finally extension at 72°C for 7 min. The amplified products were purified and sequenced on ABI 3730xl DNA Sequencer by Beijing Tianyi Huiyuan Biotechnology Co., Ltd., Beijing, China.

Identification of Beijing Lineage in 65 *Mycobacterium tuberculosis* Isolates

An improved multiplex PCR method was used to identify the Beijing genotype from the non-Beijing lineage in the 65 *M. tuberculosis* isolated from the case group in this study.[21] Two pairs of PCR primers were designed according to the reference literature.[21] Beijing was ACCGAGCTGATCAAACCCG and ATGGCACGGCCGACCTGAATGAACC (GenBank Accession number AF390039) and non-Beijing was GGTGCGAGATTGAGGTTCCC and TCTACCTGCA

Table 1 Information of Six SNPs in *VDR* Gene

Gene Locus	No.	SNP_ID	Alternative Nomenclature	Chromosome Position (bp)	Localization/ Consequence	Base Change
VDR: 12q13.11	1	rs2228570	Fok I	48272895	MIT	A>G
	2	rs731236	Taq I	48238757	I352I	A>G
	3	rs7975232	Apa I	48238837	Intron	C>A
	4	rs1544410	Bsm I	48239835	Intron	C>T
	5	rs3847987	–	48238068	UTR3	C>A
	6	rs739837	–	48238221	UTR3	G>T

GTCGCTTGTGC (Genebank Accession number BX842581). The lengths of the amplified fragments were 239bp and 308bp, respectively. The PCR products were verified by 1.5% agarose gel electrophoresis, and gel imager (Bio-Rad ChemiDoc XRS+ imaging system) was then used to observe the results.

Statistical Analysis

All analyses were performed by SPSS 25.0 (SPSS Inc., Chicago, IL, USA). The age was presented by median and interquartile range [M (P_{25}, P_{75})] and grouped into <45 and ≥45 years. The categorical variables were described by frequency and percentage [n (%)]. The χ^2 test or Fisher's exact test was used for categorical variables.

Hardy–Weinberg equilibrium (HWE) was tested by Excel in the control group.[22] Propensity score (PS) matching on the baseline characteristics and potential confounders implemented a 0.1 caliper logistic model with nearest neighbor 1:1 or 1:2 matching. The balance assessment was conducted by computing absolute standardized mean differences (SMDs) and SMD < 0.10 reflects good balance between groups. Linkage disequilibrium (LD) among the six SNPs was analyzed using the LD plot function of Haploview (version 4.2) and was assessed by using D' and r^2 values obtained through this software.[23] The stronger LD was defined between two SNPs with values of D' or r^2 closer to one. Haploview (version 4.2) was also used to generate haplotypes of SNPs in the blocks. A multivariable logistic analysis was used to study the relationships of alleles, genotypes, haplotypes and demographic factors with tuberculosis status as applicable. The statistical significance was established at $P < 0.05$.

Results

Demographic Characteristics

Two hundred and twenty-one tuberculosis patients as the case group and 363 healthy staff as the control group were included in this study. The differences in gender and age between the two groups were statistically significant (both P values <0.05). Studies have shown that gender and age are also factors influencing the incidence of tuberculosis.[1,24] In the following analysis, multivariable logistic regression analysis and PS were used to control the effect of these factors on the real relationship between *VDR* polymorphisms and tuberculosis susceptibility. Other detailed demographic characteristics of participants are shown in Table 2.

HWE Testing

The results showed that the distributions of *VDR* Fok I, Taq I, Apa I, Bsm I, rs3847987 and rs739837 genotypes in the control group conformed to the HWE in genetic inheritance ($P > 0.05$), which suggested that the control group is representative of the studying population, as shown in Table 3.

Association Analysis of *VDR* Gene Polymorphisms and Susceptibility to Tuberculosis

Univariate Analysis on the Associations Between the Alleles and Genotypes of Four Loci and Susceptibility to Tuberculosis

For the whole subjects, the results of the univariate analysis showed that none of the alleles or genotypes of six loci were statistically associated with susceptibility to tuberculosis (Table 4), whilst Taq I genotype distribution showed a potent relationship with tuberculosis status ($P = 0.087$).

We also used PS with nearest neighbor 1:1 matching to control the effect attributed to age and gender between the two groups, and 105 subjects were included in each group after matching. Among these subjects, the SMD values of each covariate (age and gender) were less than 0.10, indicating a good balance between the groups and good propensity score matching. The results of univariate analysis among subjects after matching showed no statistically significant differences in the distribution of *VDR* gene polymorphisms between the two groups (Table 4).

The Linkage Disequilibrium and Haplotype Analysis

The D' and r^2 values acquired by LD analysis with Haploview among the six SNPs in *VDR* were shown in Table 5, and one block involving rs3847987 and rs739837 was found (Figure 1). Haplotype analysis of the two selected SNPs was

Table 2 Demographic Characteristics of the Research Objects

Demographic Characteristics		Cases		Controls		z/χ^2	*P*
		No.	Proportion (%)	No.	Proportion (%)		
Age (years)	$M(P_{25}–P_{75})$	49 (33.50–65.00)		34 (30.00–44.50)		5.045[a]	<0.001
	<45	104	42.4	280	75.4	55.182	<0.001
	≥45	117	57.6	83	24.6		
Gender	Male	112	49.4	112	29.3	22.834[b]	<0.001
	Female	109	50.6	251	70.7		
Nation	Han	93	36.5	112	34.0	–	–
	Uyghur	142	55.7	178	54.1		
	Kazakh	10	3.9	20	6.1		
	Hui	8	3.1	15	4.6		
	Other nationalities	2	0.8	4	1.2		
Marriage	Unmarried	46	16.8	89	17.3	–	–
	Married	163	77.2	263	78.4		
	Divorced/Widowed	12	6.0	11	4.3		
Education	Illiteracy	27	13.5	21	2.3	–	–
	Elementary/Junior high school	140	71.8	49	30.8		
	High school/Technical secondary school	32	8.8	33	9.0		
	College/Undergraduate	22	5.9	251	54.1		
	Master's degree and above	0	0.0	9	3.8		
Occupation	Worker/Farmer	147	77.1	–	–	–	–
	Civil servants	8	2.4	–	–		
	Teacher	3	1.2	–	–		
	Student	17	7.6	–	–		
	Service industry	3	2.4	–	–		
	Others	43	9.4	–	–		
Habitation	Kashgar area	70	39.4	–	–	–	–
	Aksu area	114	34.7	–	–		
	Hotan area	30	18.2	–	–		
	Kizilsu area	4	1.8	–	–		
	Bazhou area	3	1.8	–	–		

Note: [a]Wilcoxon rank sum test. [b]Chi square test.

also performed using Haploview. A total of three haplotypes were generated; however, none of which were found to be associated with a significantly increased risk of tuberculosis (Table 6).

Multivariable Logistic Regression Analysis

In the present study, we only found that Taq I genotype distribution showed a potent relationship with tuberculosis status ($P = 0.087$) on the whole subject, and further combined factors of gender and age for the multivariable logistic regression analysis. The results did not reveal an association between Taq I polymorphisms and susceptibility to tuberculosis (Table 7).

The Relationship Between Host's *VDR* Polymorphisms and Mycobacterial Lineage

Previous reports show that the host and the pathogen may have co-adaptation. The host's susceptibility to tuberculosis may also depend on the interaction between the host and mycobacterial lineages.[25–27] In the present study, we classified the patient's *M. tuberculosis* isolates as Beijing and non-Beijing lineages, and then analyzed the relationship between polymorphisms of *VDR* gene and the lineages of *M. tuberculosis* that the host infected with. Here, we also used PS with nearest neighbor 1:2 matching to control the imbalance of age and gender between the groups and got a SMD values less than 0.10, finally, 56 subjects in the control group were included to perform analysis.

Table 3 The Distributions of the *VDR* Genotypes and HWE Testing in the Control Group

Locus	Genotyping Rate* (%)	Genotype	Controls (n=363)		χ^2	*P*
			Actual Frequency	Theoretical Frequency		
Fok I	98.0	AA	188	193	1.58	0.45
		AG	153	143		
		GG	22	27		
Taq I	96.6	AA	277	277	0.01	1.00
		AG	80	80		
		GG	6	6		
Apa I	97.3	CC	151	155	1.04	0.60
		CA	173	164		
		AA	39	44		
Bsm I	97.3	CC	269	269	0.01	1.00
		CT	87	87		
		TT	7	7		
rs3847987	94.6	CC	228	232	1.45	0.49
		CA	124	117		
		AA	11	14		
rs739837	96.0	GG	149	153	1.05	0.59
		GT	174	165		
		TT	40	45		

Note: *The rate of the available genotyping samples to total samples.

Table 4 Univariate Analysis of *VDR* Gene Polymorphisms and Susceptibility to Tuberculosis

SNP	Pre-PSM				Post-PSM			
	TB Cases (n/%)	Controls (n/%)	χ^2	*P*	TB Cases (n/%)	Controls (n/%)	χ^2	*P*
Fok I								
AA	120	188	0.916	0.633	53	48	3.443	0.179
AG	85	153			43	39		
GG	16	22			9	18		
A	325	529	0.062	0.804	149	135	2.131	0.144
G	117	197			61	75		
Taq I								
AA	140	260	1.446	0.087	83	79	2.580	0.275
AG	77	100			17	24		
GG	4	3			5	2		
A	375	634	1.444	0.229	183	182	0.021	0.885
G	67	92			27	28		
Apa I								
CC	89	151	1.806	0.405	46	41	0.948	0.623
CA	100	173			43	50		
AA	32	39			16	14		
C	278	475	0.768	0.381	135	132	0.093	0.761
A	164	251			75	78		
Bsm I								
CC	172	269	2.663	0.264	82	75	2.612	0.271
CT	42	87			18	27		
TT	7	7			5	3		
C	386	625	0.364	0.546	182	177	0.479	0.489
T	56	101			28	33		

(*Continued*)

Table 4 (Continued).

SNP	Pre-PSM				Post-PSM			
	TB Cases (n/%)	Controls (n/%)	χ^2	*P*	TB Cases (n/%)	Controls (n/%)	χ^2	*P*
rs3847987								
CC	132	228	0.811	0.667	60	59	1.485	0.476
CA	80	124			40	44		
AA	9	11			5	2		
C	344	580	0.707	0.401	160	162	0.053	0.818
A	98	146			50	48		
rs739837								
GG	95	149	1.122	0.571	42	38	0.480	0.787
GT	97	174			49	54		
TT	29	40			14	13		
G	287	472	0.001	0.977	133	130	0.092	0.762
T	155	254			77	80		

Notes: [a]Variables with *P*-value less than 0.1 in the population before propensity scoring were selected to enter multivariable regression analysis.

Table 5 Linkage Disequilibrium Test for SNPs of *VDR* Gene

SNP	Fok I	Taq I	Apa I	Bsm I	Rs3847987	Rs739837
Fok I	–	0.742	0.643	0.384	0.725	0.011
Taq I	0.96	–	0.814	0.251	0.853	0.001
Apa I	0.887	0.92	–	0.165	0.968	0.007
Bsm I	0.886	0.864	0.642	–	0.208	0.011
Rs3847987	0.895	0.96	1.0	0.706	–	0.008
Rs739837	1.0	0.339	1.0	1.0	1.0	–

Note: The lower left indicates the D' value and the upper right indicates the r^2 value.

Lineage Identification of *Mycobacterium tuberculosis*

Sixty-five isolates of *M. tuberculosis* were collected from the case group, of which 32 (49.2%) were Beijing lineage and 33 (50.8%) were non-Beijing lineage.

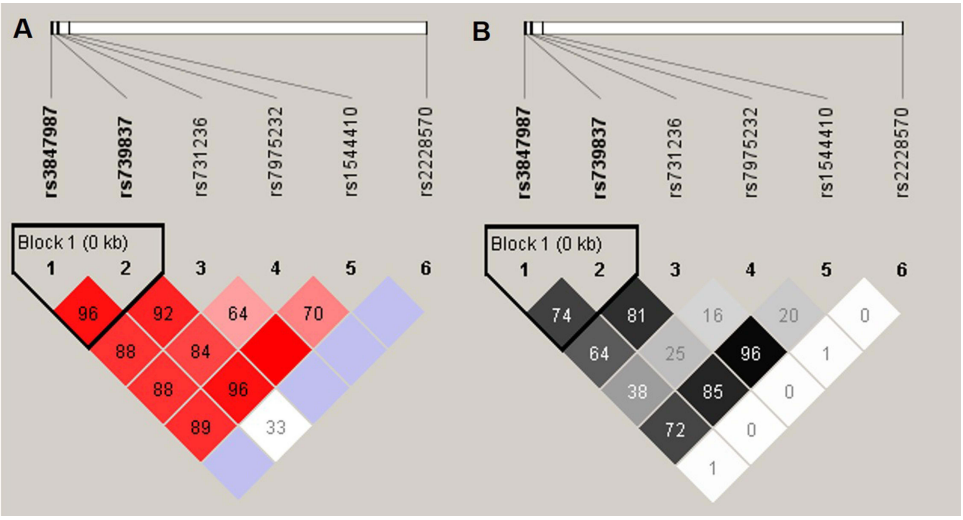

Figure 1 Linkage disequilibrium test for SNPs of *VDR* gene. (**A**) D' values between each two loci, and (**B**) r^2 values between each two loci.

Table 6 Haplotype Distributions of rs3847987 and rs739837 in the Case and Control Groups After PS

Haplotype	TB Cases (n/%)	Controls (n/%)	χ^2	*P*	OR (95% CI)
CC	133.6(63.9)	134.3(64.4)	0.004	0.948	0.987(0.661–1.473)
TG	44.8(21.4)	40.1(19.2)	0.340	0.560	1.152(0.715–1.857)
TC	30.5(14.6)	34.0(16.3)	0.182	0.670	0.891(0.525–1.514)

Table 7 Multivariable Logistic Regression Analysis of *VDR* Gene Polymorphisms and Susceptibility to Tuberculosis Among the Whole Subjects

Factors	OR (95% CI)	*P*
Age (years)		
<45	1	
≥45	0.248(0.150–0.409)	<0.001
Gender		
Male	1	
Female	2.026(1.231–3.334)	0.005
Taq I		
AA	1	
AG	1.257(0.340–4.647)	0.732
GG	2.318(0.607~8.856)	0.219

The Association Between Polymorphisms of *VDR* Gene and Lineages of *Mycobacterium tuberculosis*

As shown in Table 8, the distributions of the genotypes and alleles of Fok I, Taq I, Apa I, Bsm I, rs3847987 and rs739837 in the *VDR* gene between patients infected with Beijing lineage and non-Beijing lineage and control group were not found to be statistically different. Further haplotype analysis was used, and the results showed that there were linkage disequilibria among the two SNPs rs3847987 and rs739837 (Supplemental Figures S1–S3). Although no statistically significant associations were observed in haplotype, they constitute and tuberculosis susceptibility (Supplemental Tables S1–S3).

Table 8 The Associations Between Polymorphisms of *VDR* Gene and Lineages of *M. tuberculosis* in the Population After PS

Factors		Beijing Lineage (n/%)	Non-Beijing Lineage (n/%)	Controls (n/%)	*P* (Beijing vs Non-Beijing)	*P* (Beijing vs Controls)	*P* (Non-Beijing vs Controls)
Age (years)	<45	11(57.9)	8(42.1)	22(39.3)	0.422	0.647	0.147
	≥45	21(45.7)	25(54.3)	34(60.7)			
Gender	Male	17(54.8)	14(45.2)	26(46.4)	0.388	0.545	0.714
	Female	15(44.1)	19(55.9)	30(53.6)			
Fok I	AA	4(12.5)	5(15.2)	9(16.1)	0.953[a]	0.870	0.935
	AG	14(43.8)	14(42.4)	25(44.6)			
	GG	14(43.8)	14(42.4)	22(39.3)			
	A	22(34.4)	24(36.4)	43(38.4)	0.813	0.595	0.787
	G	42(65.6)	42(63.6)	69(61.6)			
Taq I	AA	23(71.9)	24(72.7)	38(67.9)	0.484[a]	0.250[a]	0.786
	AG	9(28.1)	8(24.2)	15(26.8)			
	GG	0(0.0)	1(3.0)	3(5.4)			
	A	55(85.9)	56(84.8)	91(81.3)	0.861	0.426	0.541
	G	9(14.1)	10(15.2)	21(18.8)			

(*Continued*)

Table 8 (Continued).

Factors		Beijing Lineage (n/%)	Non-Beijing Lineage (n/%)	Controls (n/%)	*P* (Beijing vs Non-Beijing)	*P* (Beijing vs Controls)	*P* (Non-Beijing vs Controls)
Apa I	CC	13(40.6)	13(39.4)	14(25.0)	0.837	0.304	0.524
	CA	14(43.8)	13(39.4)	32(57.1)			
	AA	5(15.6)	7(21.2)	10(17.9)			
	C	40(62.5)	39(59.1)	60(53.6)	0.691	0.250	0.474
	A	24(37.5)	27(40.9)	52(46.4)			
Bsm I	CC	23(71.9)	22(71.0)	34(60.7)	0.484[a]	0.128[a]	0.683[a]
	CT	9(28.1)	8(25.8)	18(32.1)			
	TT	0(0.0)	1(3.2)	4(7.1)			
	C	55(85.9)	52(83.9)	86(76.8)	0.746	0.143	0.269
	T	9(14.1)	10(16.1)	26(23.2)			
rs3847987	CC	21(65.6)	19(57.6)	30(53.6)	0.522	1.162	0.199
	CA	8(25.0)	10(30.3)	19(33.9)			
	AA	3(9.4)	4(12.1)	7(12.5)			
	C	50(78.1)	48(72.7)	79(70.5)	0.475	0.274	0.755
	A	14(21.9)	18(27.3)	33(29.5)			
rs739837	GG	16(50.0)	15(45.5)	30(53.6)	0.701	1.838	0.973
	GT	14(43.8)	14(42.4)	18(32.1)			
	TT	2(6.20)	4(12.1)	8(14.3)			
	G	46(71.9)	44(66.7)	78(69.6)	0.520	0.755	0.680
	T	18(28.1)	22(33.3)	34(30.4)			

Note: [a]Acquired by Fisher's exact test, while others acquired by χ^2 test.

Discussion

This is the first report on the interaction between the *VDR* gene polymorphisms of population of China and the lineage of *M. tuberculosis*. Previous studies show that variants in human genes *TLR2*,[28] *SLC11A1*,[29] *CD53*,[30] *G57E*[31] and *LAMP1*[32] have correlations with *M. tuberculosis* lineage, implying co-evolution between humans and *M. tuberculosis*. In this study, we tried to find correlations between the Fok I, Taq I, Apa I, Bsm I, rs3847987 or rs739837 polymorphisms of *VDR* gene of population in southern Xinjiang and the occurrence of tuberculosis or the lineages of *M. tuberculosis* infected. However, no significant link was found.

With the improvement and availability of gene sequencing technology, a large number of disease-related susceptibility genes have been discovered. Genes related to tuberculosis susceptibility have been reported continuously through genome-wide association studies (GWAS) and hot SNP correlation studies, including human leukocyte antigen (*HLA*) genes and non-HLA genes like *NRAMP1, VDR*, *SP110,* etc. A study among twins shown that the consistency of tuberculosis in identical twins is significantly higher than that in fraternal twins,[33] indicating that genetic susceptibility is one of the important risk factors for developing active tuberculosis, even within the same race. However, reports on the genes or SNPs associated with making human beings susceptible to tuberculosis development were inconsistent or contradictory due to the geographical restrictions and ethnic differences.[5,25,34] There were few reports on the susceptibility genes of tuberculosis in population from Xinjiang, China.[9,10,35]

Observational studies in recent years have found that vitamin D has an anti-tuberculosis effect in vitro, with reduced proliferation of *M. tuberculosis* in vitamin D-treated macrophages.[36] The mechanism may be that Toll-like receptors on macrophages are activated by *M. tuberculosis*, which in turn induces upregulation of vitamin receptor transcription and enhances CYP27B1 expression. CYP27B1 promotes increased synthesis of $1,25(OH)_2 D_3$, which in combination with VDR is involved in regulating the expression of various genes, including the secretion of some cytokines and the expression of antimicrobial peptides, thereby increasing the killing power of macrophages against *M. tuberculosis*.[37,38] However, the diversity of genetic backgrounds and survival environments in different populations may lead to a diversity of various genotypes and allele frequencies of *VDR*, and therefore the role of *VDR* gene polymorphisms in different populations varies.

In the present study, 221 tuberculosis patients and 363 healthy controls from southern Xinjiang were selected to explore the polymorphisms of *VDR* associated with tuberculosis. The results showed that the distribution frequencies of alleles and genotypes of Fok I, Taq I, Apa I, Bsm I, rs3847987 and rs739837 in patients were not statistically different from that of the control group, which was inconsistent with the previously reported results.[5,6,39] For example, Sharma et al reported that Bsm I polymorphism appeared associated with the tuberculosis in tribes, castes and Muslims of central India, whereas, Taq I, Fok I and Apa I polymorphisms revealed association in general population and Muslims only.[40] The report in the Brazilian population found that Taq I-TT genotypes, Fok I-CC/(CC+CT) and Bsm I-GG genotypes were significantly associated with tuberculosis susceptibility, of which the homozygotes of Taq I-TT and Bsm I-GG both have approximately twofold increase in risk ratio than other genotypes.[5] Similarly, Taq I-TT was also found to be a risk factor in the population of Andhra Pradesh in India,[6] and the result is opposite in northern India.[39] However, the study on the relationship between *VDR* gene rs3847987, rs739837 polymorphism and pulmonary tuberculosis has not been reported. Ganmaa et al conducted a cross-sectional study of 9810 children and found that serum vitamin D deficiency was a potential risk factor for tuberculosis,[41] while Ruiz-Tagle et al found that hypovitaminosis D had little effect on the *M. tuberculosis* infection.[42] Another report showed that active tuberculosis children owned lowed vitamin D and *VDR* mRNA expression levels and increased *VDR* DNA methylation than the healthy controls, so the *VDR* hypermethylation may be involved in the impairment in the *VDR*-mediated cytolytic and antimicrobial effector cell response in pediatric TB disease.[43] Future studies can explore the roles of DNA methylation and its interaction with SNPs in *VDR* in adult tuberculosis.

The present study showed that the proportion of Beijing lineage was 50.8% (33/65), which was consistent with previous studies from Xinjiang, China.[15–17,44] It has been reported that human pathogens have geographically structured population genetics and each of the six phylogeographical lineages (lineage 1 to 6) of *M. tuberculosis* were associated with representative, sympatric human populations.[45,46] Lineage 1, 2, 3, and 4 of *M. tuberculosis* isolates were prevailing in China based on the database of the National Survey of Drug-Resistant Tuberculosis in 2007 and reported that Lineage 2 accounted for 75% of all strains.[44] Lineage 2 (or East Asian lineage) which show high prevalence in East-Asian countries is majorly composed of Beijing family which attract attention by their hyper-virulence in laboratory models, their recent dissemination in human populations, and their association with drug resistance.[47,48] Several studies have conducted genotyping analysis on *M. tuberculosis* isolates from Xinjiang, and the results show that the proportions of Beijing lineage isolates range from 42% to 60%,[15–17,44] which was lower than the national average of 73.9% in China.[46]

During the analysis on association between polymorphisms of *VDR* gene and lineages of *M. tuberculosis*, we grouped tuberculosis patients according to information on the genealogy of the infecting pathogens and no significance associations were found. We also combined the haplotypes to help locate causative genes or regions with tuberculosis or *M. tuberculosis* infection more precisely. Haplotypes are linear arrangements of alleles with a tendency to be inherited as a whole. In the present study, we found that two out of six loci of the *VDR* gene formed one haplotype block, and the generated haplotypes were not observed to be associated with susceptibility to tuberculosis or the lineages of *M. tuberculosis* infected. Omae et al performed a *M. tuberculosis* lineage-based GWAS with *CD53* gene and found that two SNPs (rs1418425 and rs1494320) are risk factors for the old age tuberculosis onset infected with strains of non-Beijing lineage, whilst no correlation were found in old age cases infected with Beijing lineage, they speculated that the genetic risk of host susceptibility is related to the lineage of infected strains.[30] Müller et al evaluated two geographically distinct cohorts (South African population composed of 947 participants and the Ghanaian population composed of 3311 participants) and found that 32 SNPs were statistically significantly associated with risk for infection with different types of the *M. tuberculosis* complex in the Ghanaian cohort, whilst no correlation were found in South African cohort.[26]

There were several limitations in the present study, including that we enrolled healthy staffs who worked in hospitals for the control group instead of enrolling individuals in communities and *M. tuberculosis* isolates were only acquired from part of cases, improvements should be taken in the future studies. Second, there were still some factors without being consideration in our studies such as the expression levels of *VDR*, vitamin D levels of population and drug susceptibilities of isolates, which are essential to understand the association between *VDR* gene and susceptibility to tuberculosis. Third, the present study did not evaluate the probability of latent tuberculosis infectious disease with these SNPs progressing to tuberculosis. In the future studies, all of these factors should be improved or strengthened.

Conclusions

In conclusion, the present study showed no relation between *VDR* gene polymorphisms and tuberculosis susceptibility in the population from Xinjiang, China. Much evidence has shown that the evolution of *M. tuberculosis* in the host was affected by the immunity of the host, which provides hints of focusing on the immune-related genes to find genes for tuberculosis susceptibility. So, further researches are needed to clarify the associations between *VDR* and tuberculosis susceptibility.

Abbreviations

CTAB, Cetyltrimethylammonium bromide; EDTA, Ethylene diamine tetraacetic acid; GWAS, Genome-wide association studies; HIV, Human immunodeficiency virus; HLA, Human leukocyte antigen; HWE, Hardy–Weinberg equilibrium; LD, Linkage disequilibrium; PS, Propensity score; SMD, Standardized mean differences; SNPs, Single-nucleotide polymorphisms; VDR, Vitamin D receptor; WHO, World Health Organization.

Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

This study was in line with the Declaration of Helsinki and obtained approval (XJMU8HEC-20161215) from the Ethics Committee of The Eighth Affiliated Hospital of Xinjiang Medical University. All methods were performed in accordance with the relevant guidelines and regulations. Written informed consent was obtained from individual or guardian participants.

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Disclosure

We have no conflicts of interest to declare.

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