MAJOR ARTICLE

Vitamin D Receptor Polymorphisms and Susceptibility to Tuberculosis in West Africa: A Case-Control and Family Study

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Vitamin D receptor *(VDR)* gene polymorphisms have been implicated in susceptibility to tuberculosis (TB), but reports have been inconsistent. We genotyped the *VDR* single-nucleotide polymorphisms (SNPs) *Fok*I, *Bsm*I, *Apa*I, and *Taq*I in 1139 case patients and control subjects and 382 families from The Gambia, Guinea, and Guinea-Bissau. The transmission-disequilibrium test on family data showed a significant global association of TB with SNP combinations *Fok*I-*Bsm*I-*Apa*I-*Taq*I and *Fok*I-*Apa*I that were driven by the increased transmission to affected offspring of the *Fok*I F and *Apa*I A alleles in combination. The *Apa*I A allele was also transmitted to affected offspring significantly more often than expected. Case-control analysis showed no statistically significant association between TB and *VDR* variants. *Bsm*I, *Apa*I, and *Taq*I showed strong linkage disequilibrium. The significance of the family-based associations found between TB and *Fok*I-*Bsm*I-*Apa*I-*Taq*I and the FA haplotype supports a role for *VDR* haplotypes, rather than individual genotypes, in susceptibility to TB.

An improved understanding of the pathogenesis of tuberculosis (TB) and effective treatment for it are significantly influenced by our ability to untie the effects of host genetic and environmental factors in response to TB. A recent innovative approach involved joint investigation of these factors in 3 West African countries by use of a combined study design [1]. In an accompanying paper, Bennett et al. [2] reviewed evidence that host genetic factors play a role in susceptibility to TB. The present article is a component of the multicenter study described above and reports the investigation of the effect of vitamin D receptor *(VDR)* gene polymorphisms, as a host genetic factor, on the development of TB.

The development of TB in humans is a 2-stage process through which a susceptible person exposed to an infectious case first becomes infected and, after an interval of years or decades, may later develop the disease [3]. The large majority of individuals infected with *Mycobacterium tuberculosis* have no recognizable ill effects. In some individuals, however, the primary infection is not contained and goes on to cause substantial tissue damage and clinical disease, either locally or by hematogenous spread of bacilli from the initial lung site to other organs of the body. This primary disease is more likely to occur in children, some weeks or months

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after the primary infection, but also in HIV-infected individuals. In most infected individuals, however, tubercle bacilli remain dormant, and overt pulmonary TB disease usually arises many years after primary infection, either through the breakdown of a localized lesion in the lungs (endogenous reactivation) or through reinfection from exposure to infectious TB [4]. The relative contribution of reactivation and reinfection is likely to depend on the epidemiological context, but it is generally accepted that, in populations at high risk for infection, reinfection might be a major contributor to the overall rate of TB in adults, whereas, in populations at low risk for infection, most cases of postprimary disease in adults probably result from reactivation [4, 5].

Among persons who are exposed to persons with infectious TB, the risk of becoming infected is primarily determined by the combined action of the infectivity of the source case, the intensity of exposure of the susceptible person to that case, and his/her susceptibility to infection [4]. Factors reported to influence the risk of infection include age, sex, crowding, socioeconomic conditions, urbanization, race/ethnic group, and HIV status [6]. The risk of developing disease after infection is strongly dependent on age and time, but any condition that modifies the balance established in the body between the tubercle bacilli and host immune defenses can have an effect on the risk of developing disease. Thus, factors that have been shown to influence this balance include age, sex, HIV infection, immunosuppressive treatment, diabetes, malnutrition, alcoholism, and bacille Calmette-Guérin vaccination [7]. In addition, there is increasing evidence that genetic factors, in part, determine differences in host susceptibility to infection with mycobacteria and that they might contribute to the pattern of clinical disease [8].

Differential susceptibility to TB is most likely determined by several genes [9, 10], because genomewide screening has identified no single, strongly linked marker [11, 12]. In support of this, candidate gene–based case-control studies found several immunogenetic polymorphisms with a moderate effect on risk of clinical TB [9, 10], including allelic variants of *VDR* [13]. VDR is synthesized in monocytes and activated T and B lymphocytes [14], whereas its ligand, the active metabolite of vitamin D (1,25 dihydroxy vitamin D [calcitriol]), is produced in the kidney and by activated monocytes and macrophages, in particular in granuloma [15, 16]. Through its interaction with vitamin D, the retinoid X receptor (RXR), and the vitamin D response element (VDRE), VDR exerts several immunomodulatory effects [14]. These include the activation of monocytes and cell-mediated immunity, modulation of the Th1-Th2 host immune response, suppression of lymphocyte proliferation, and restriction of *M. tuberculosis* survival in macrophages [17–19].

In 1999, Bellamy et al. [13] reported that the tt genotype of *VDR,* which is often associated with lower bone mineral density, was found less frequently in patients with pulmonary TB in The Gambia. In the same population, tt was found to be negatively associated with persistent hepatitis B virus infection [13]. A subsequent study found tt to be associated with tuberculoid leprosy and TT with lepromatous leprosy in India [20], which implicates that allelic variants of *VDR* influence a Th1-Th2 shift in the host immune response that determines leprosy type. Since then, several case-control studies have reexamined the association of *VDR* polymorphisms with diseases of mycobacterial origin in different populations [21–26], but different risk alleles have been reported or no independent association was found (summarized in Discussion). Particular haplotypes of *VDR,* which harbors alleles that affect both mRNA expression (promoter and the 3′ untranslated region [UTR]) and protein function (coding region), were proposed to reflect functional effects more accurately [27] and may associate more consistently with disease.

In the present study, it was proposed that *VDR* haplotypes, rather than individual alleles or genotypes, are responsible for associations between TB and *VDR* variants. The objective was to identify susceptibility haplotypes in West African populations by use of a family-based approach, which is robust to population stratification, and to verify the role that variants play in *VDR* haplotypes that were previously associated with TB in case-control studies. We genotyped the *VDR* single-nucleotide polymorphisms (SNPs) *Fok*I, *Bsm*I, *Apa*I, and *Taq*I in 382 families and 1139 case-control subjects from 3 West African countries: The Gambia, Guinea, and Guinea-Bissau. Results from the family-based study support a role for *VDR* haplotypes in susceptibility to TB in West Africans.

PATIENTS AND METHODS

Patients. The study was conducted in 3 West African countries (The Gambia, Guinea, and Guinea-Bissau) according to a protocol described elsewhere [1, 2]. The present study did not use samples previously collected, as documented by Bellamy et al. [13]. Patients with newly detected TB who were >15 years old who had been living at the same address for >3 months were eligible for inclusion in the study. Pulmonary TB was confirmed by 2 consecutive sputum smears positive for acid-fast bacilli and/or a positive sputum culture. Patients were fully clinically examined, and an anterioposterior chest radiograph was systematically obtained to characterize disease severity. Matched healthy community control subjects were recruited from the vicinity of the patient with TB, as described elsewhere [1]. Where possible, the father, mother, and full siblings of the patient with TB were also recruited, and those patients with TB whose family members had been genotyped were included in an independent family-based association analysis ($n = 382$). Initially, 436 families were included in the study, for a total of

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1413 individuals. Inheritance checking was continuously done by genotyping 4 microsatellites and >50 SNPs, including the *VDR* SNPs. Samples were removed from the transmission-disequilibrium test (TDT) analysis if genotypes showed noninheritance at >2 loci, as genotyped on separate occasions, and 94 individuals were excluded from the study. The remaining case patients ($n = 417$) and healthy control subjects ($n = 722$) formed the case-control study, which had 80% power to detect an association (at $P = .05$) for a genotype of 6%–88% frequency and an odds ratio (OR) of ⩾2 or 22%–70% frequency and an OR of ⩾1.5. Table 1 shows demographic and clinical data for both studies.

DNA was extracted from venous blood by use of the Nucleon BACC2 DNA extraction kit (Nucleon Bioscience) and standard phenol-chloroform procedures. After samples were transported to the University of Oxford, DNA concentrations were determined by use of the PicoGreen kit (Molecular Probes), and working stocks of DNA samples at 10 ng/μL were prepared. Informed consent was obtained from patients or their parents or guardians. Ethical approval was provided by the joint Gambian Government/Medical Research Council Ethical Committee, Ministry of Public Health (MINSAP, Guinea-Bissau), and National Ethics committee, Ministry of Health, Conakry, République de Guinée. Human-experimentation guidelines of these ministries were followed.

Genotyping. The human *VDR* is located on 12q12–14 and contains 16 exons that encode the 5′ UTR (exons IA–IG), the DNA-binding domain (exons II and III), and the overlapping ligand-binding and heterodimerization domains (exons VI–IX) [28, 29]. *VDR* contains >25 known polymorphisms, whereas >100 are expected on the basis of the observed genomewide frequency of SNPs [27]. The most commonly studied variants include a *Fok*I restriction fragment–length polymorphism (RFLP) in exon II (alleles F/f or nucleotides C/T), *Bsm*I (B/b or nucleotides T/C) and *Apa*I (A/a or nucleotides T/G) variants in intron VIII, and a *Taq*I (T/t or nucleotides T/C) variant in exon IX, with lowercase patient alleles indicating the presence of restriction sites. *Bsm*I, *Apa*I, and *Taq*I are in strong linkage disequilibrium (LD) with a singlet (A) repeat in the 3′ UTR, which results in short (S) or long (L) alleles. DNA samples were genotyped for the *VDR* SNPs *Fok*I, *Bsm*I, *Apa*I, and *Taq*I, because they were relatively informative and have been previously associated with mycobacterial infections [13, 21–26, 30]. All SNPs, with the exception of *Fok*1, were genotyped by use of the ligation detection reaction (LDR) [31]. LDR products were analyzed by use of a capillary electrophoresis ABI 3700 machine and the Genotyper software package (Applied Biosystems). *Fok*1 was genotyped by use of allele-specific polymerase chain reaction (PCR) and control amplification of the third intron of HLA-DRB1, followed by resolution on agarose gels [32]. Genotyping was verified and confirmed by sequencing of 96 samples. No discrepancies were found, and no samples were excluded. The sequences of primers used for LDR and allele-specific PCR are summarized in table 2. PCRs of the region containing *Bsm*I, *Apa*I, and *Taq*I was performed by use of 3 consecutive cycles with annealing temperatures of 71°C, 64°C, and 55°C repeated 5, 21, and 4 times each. PCR of the *Fok*I RFLP was performed by use of 1 cycle with an annealing temperature of 58°C repeated 35 times. The LDR ligation temperature was 72°C for *Bsm*I, *Apa*I, and *Taq*I.

Statistical analysis. In the case-control study, testing for association at each polymorphism was initially performed by use of a 3×2 χ^2 test with 2 *df*. Both the case patients and the control subjects were also tested for Hardy-Weinberg equilibrium (HWE) by use of a 3×2 χ^2 test with 1 *df*. Because the aim of the study [1, 2] was to identify both genetic and nongenetic risk factors for TB, the case patients and control subjects were only matched by age [1]. There are therefore significant differences between case patients and control subjects for variables such as sex, country, ethnic group, and HIV status. To control for the potential confounding effects of age, sex, country, ethnic group, and HIV status, multivariate logistic regression was performed by use of STATA (version 7; Stata). Where these factors were not found to have a significant effect, they were dropped from the model. Family-based association was analyzed by the TDT [33] by use of the *Transmit* program [34]. The TDT is robust to ethnic stratification, whereas *Transmit* allows for missing parents and the analysis of multilocus haplotypes. Rare haplotypes with frequencies <2% were combined in the analysis. For LD analysis, maximum-likelihood estimates of pairwise LD were obtained by use of LDMAX within the GOLD package [35]. All case patients and control subjects were included in this analysis. For the sake of simplicity and for all analyses, results for individual SNPs are shown first.

RESULTS

Case-control study. χ^2 analysis of the genotype frequencies for *VDR* polymorphisms in case patients with TB and control subjects from The Gambia, Guinea, and Guinea-Bissau, alone or in combination, showed no significant association with TB (table 3). To investigate possible associations between the severity of disease and *VDR* polymorphisms, analysis was restricted to case patients with severe TB ($n = 125$), defined as ⩾4 lung zones affected and the presence of cavitations on radiographs (table 1, bottom left). Again, no significant association between any genotype and TB was observed (table 4). In all instances (i.e., case patients, control subjects, and different population groups), SNP genotypes were in HWE, and no confounding effect of sex, age, country, ethnicity, or HIV status was detected (data not shown). The HIV-positivity rate in control subjects was 6.8%, and that in case patients was 12.5% (table 1). Although the numbers were very small, subgroup

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Table 1. **Demographic and clinical details of participants in the case-control and family studies.**

Characteristic	Case-control study: Patients with TB (n = 417)	Case-control study: Healthy community control subjects (n = 722)	Case patients with TB and family members included in the TDT
Country			
The Gambia	175 (42.0)	350 (48.5)	218 (57.1)
Guinea	157 (37.6)	199 (27.6)	74 (19.4)
Guinea-Bissau	85 (20.4)	173 (24.0)	90 (23.6)
Ethnic group			
Mandinka	82 (19.7)	155 (21.5)	
Fula	104 (24.9)	144 (19.9)	
Sousou	61 (14.6)	77 (10.7)	
Jola	30 (7.2)	69 (9.6)	
Wolof	17 (4.1)	35 (4.8)	
Manjago	21 (5.0)	49 (6.8)	
Balanta	23 (5.5)	38 (5.3)	
Serere	13 (3.1)	23 (3.2)	
Pepel	6 (1.4)	33 (4.6)	
Other	54 (12.9)	92 (12.7)	
Unknown	6 (1.4)	7 (1.0)	
Sex			
Male	280 (67.1)	380 (52.6)	262 (68.6)
Female	137 (32.9)	342 (47.4)	120 (31.4)
Age, mean (SD), years	34.7 (12.60)	32.6 (10.97)	29.1 (10.6)
HIV status			
Negative	356 (85.4)	617 (85.5)	337 (88.2)
Positive	52 (12.5)	49 (6.8)	40 (10.5)
Unknown	9 (2.2)	56 (7.8)	5 (1.3)
Zones affected			
0–3	145 (34.8)	…	138 (36.1)
4–6	210 (50.4)	…	182 (47.6)
Unknown	62 (14.9)	…	62 (16.2)
Cavitation present			
Yes	174 (41.7)	…	158 (41.4)
No	184 (44.1)	…	162 (42.4)
Unknown	59 (14.1)	…	62 (16.2)
Severe (4–6 zones and cavitation)	125 (30.0)	…	110 (69.6)
Family composition			
2 parents			73 (19.1)
1 parent, 0 siblings			63 (16.5)
1 parent, 1 sibling			54 (14.1)
1 parent, >1 sibling			70 (18.3)
0 parents, 1 sibling			59 (15.4)
0 parents, >1 sibling			63 (16.5)

NOTE. Data are no. (%), unless otherwise indicated. Participants in the case-control study were genotyped for at least 1 polymorphism ($n = 1139$). A total of 382 patients with tuberculosis (TB), coming from families of different composition, were used for the transmission-disequilibrium test (TDT).

analysis for HIV-positive patients was done by comparing HIV-positive patients with TB versus HIV-positive control subjects and HIV-positive patients with TB versus all control subjects, for each SNP (all 3 genotypes). All tests showed no association (results not shown).

Family-based study. The TDT, performed by use of *Transmit*, revealed that the *Apa*I A allele was transmitted to affected offspring significantly more often than expected ($\chi^2 = 4.925$, $P = .0265$, 1 *df*) and the a allele significantly less often than expected (table 5). Observed transmissions of alleles for *Fok*I, *Bsm*I, or *Taq*I were not significantly different from expected values (table 5). However, *Apa*I did not conform to HWE in parents ($P = .038$), whereas all other SNPs did in both case patients with TB and their parents. It is possible that this contributes to the association of *Apa*I in the families; however, the deviation from HWE was of borderline significance and was not seen in any other group tested for *Apa*I. In addition to this, the frequency of the *Apa*I AA genotype in the case patients with TB from the family study (159/329 [48.3%]) was higher than that in the control subjects from the case-control study

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Table 2. The sequence of polymerase chain reaction (PCR) primers used for the analysis of single-nucleotide polymorphisms (SNPs) in the vitamin D receptor *(VDR): Bsm*I, *Apa*I, and *Taq*I by ligation detection reaction and *Fok*I by allele-specific PCR.

Type of primer	Sequence
PCR primers used for amplification of the region containing *Bsm*I, *Apa*I, and *Taq*I	
Forward	CTGGGGAGCGGGGAGTATGAAGGA
Reverse	GGGTGGCGGCAGCGGATGTA
Fluorescent allelic detecting primers	
*Bsm*I	FAM-CAAGAGCAGAGCCTGAGTATTGGGAATGT
	HEX-AAAGAGCAGAGCCTGAGTATTGGGAATGC
*Apa*I	FAM-GGGTGGTGGGATTGAGCAGTGAGGT
	HEX-AGGTGGTGGGATTGAGCAGTGAGGG
*Taq*I	FAM-TGCAGGACGCCGCGCTGATT
	HEX-AGCAGGACGCCGCGCTGATC
Common phosphorylated primer	
*Bsm*I	GCAGGCCTGTCTGTGGCCCCAGATTAAAAAAAAATA
*Apa*I	GCCCAGCTGAGAGCTCCTGTGCCTTAAAAAAAATAAAAAAAAA
*Taq*I	GAGGCCATCCAGGACCGCCTGTCATTATATAAAATATAAATATAAAAAAA
Allele-specific primers of the VDR SNP, *Fok*I[a]	
F allele	TGGCCGCCATTGCCTCC**G** (ACG)
f allele	TGGCCGCCATTGCCTCC**A** (ATG)
Consensus	AGCTGGCCCTGGCACTGA
Amplification control primers[b]	
HLA DRB1 Forward	TGCCAAGTGGAGCACCCAA
HLA DRB1 Reverse	GCATCTTGCTCTGTGCAGAT

NOTE. All primers are listed in the 5′→3′ orientation. Bold, underlined nucleotides highlight the allele specificity of the *Fok*I primers.

[a] Allele-specific primers amplify a sequence that is 78 bp in length.

[b] Control primers amplify a sequence that is 796 bp in length from the HLA region.

(266/634 [42.0%]), which shows that the association seen here cannot be entirely explained by the slight deviation from HWE seen in the parents.

To investigate whether *Apa*I or a neighboring, untested polymorphism was causative, the transmission of haplotypes was also studied. Significant global association with TB was detected for the haplotypic combination of all 4 SNPs (*Fok*I-*Bsm*I-*Apa*I-*Taq*I, $\chi^2 = 22.113$, $P = .0085$, 9 *df;* table 5). However, no individual haplotype was significantly associated. *Taq*I does not contribute significantly to the observed effect—haplotypes produced by *Fok*I, *Bsm*I, and *Apa*I were also globally associated ($\chi^2 = 15.451$, $P = .0170$, 6 *df*), primarily because of the increased transmission of FBA and decreased transmission of Fba and fBA (table 5). To further define the haplotypes that drive these associations, we examined combinations of 2 SNPs from this 3-SNP haplotype (i.e., *Fok*I-*Apa*I, *Bsm*I-*Apa*I, and *Fok*I-*Bsm*I). The strongest association was observed for *Fok*I-*Apa*I haplotypes (global $\chi^2 = 12.33$, $P = .0063$, 3 *df*), driven by increased transmission of the FA haplotype ($\chi^2 = 11.621$, $P = .0007$, 1 *df*). Other associated haplotypes (table 5) were FB ($\chi^2 = 4.355$, $P = .0369$, 1 *df*), which was transmitted to affected offspring more often than expected, and Fa ($\chi^2 = 3.917$, $P = .0478$, 1 *df*), Ba ($\chi^2 = 4.226$, $P = .0398$, 1 *df*), and fB ($\chi^2 = 4.063$, $P = .0438$, 1 *df*), which were transmitted to affected offspring significantly less often than expected. Because a *Taq*I protective association with the tt genotype was previously reported in The Gambia [13], the *Apa*I-*Taq*I haplotype was also investigated; however, no significant association was found (table 5).

LD analysis. LD analysis of the 4 *VDR* SNPs studied was conducted in the case-control samples and illustrated in figure 1. The three 3′ SNPs (*Bsm*I-*Apa*I-*Taq*I) showed significant, strong LD with each other, as has been shown in previous studies [36, 37], especially *Bsm*I-*Apa*I and *Apa*I-*Taq*I. In contrast, *Fok*I was in LD with only *Bsm*I, significantly but less strong than the LD between *Bsm*I-*Apa*I and *Apa*I-*Taq*I. The pattern of LD was the same in all 3 countries (data not shown).

DISCUSSION

In the family-based study, the TDT showed a global association of the SNP combinations *Fok*I-*Bsm*I-*Apa*I-*Taq*I and *Fok*I-*Apa*I with TB that was more significant than that to any individual SNP. This supports the proposal that haplotypes more accurately and consistently reflect associations between TB and *VDR* variants than to individual polymorphisms. The role that *VDR* polymorphisms play in susceptibility to TB in West Africa was evaluated by use of both a case-control and a family-based approach. The case-control study results provide an estimate of the association between a certain gene or allele and a given disease but suffer from potential bias due to population admixture or stratification, as is likely to exist in ethnically mixed African populations. In contrast, being performed within fam-

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Table 3. Genotype frequencies for single-nucleotide polymorphisms (SNPs) in the vitamin D receptor *(VDR)* in all patients with tuberculosis (TB) and control subjects from The Gambia, Guinea, and Guinea-Bissau combined.

SNP, genotype	Patients with TB, no. (%)	Control subjects, no. (%)	χ^2	*df*	*P*
*Fok*I			0.094	2	.954
FF	258 (62.0)	444 (61.8)			
Ff	138 (33.2)	242 (33.7)			
ff	20 (4.8)	32 (4.5)			
Total	416	718			
*Bsm*I			0.255	2	.880
bb	215 (62.7)	387 (61.0)			
bB	108 (31.5)	208 (32.8)			
BB	20 (5.8)	39 (6.2)			
Total	343	634			
*Apa*I			0.550	2	.760
aa	38 (11.1)	76 (12.0)			
aA	153 (44.6)	292 (46.0)			
AA	152 (44.3)	266 (42.0)			
Total	343	634			
*Taq*I			2.312	2	.315
tt	37 (10.8)	50 (7.9)			
tT	132 (38.5)	253 (39.9)			
TT	174 (50.7)	331 (52.2)			
Total	343	634			

ilies, the TDT is robust to population stratification and minimizes that bias, but it does not give an estimate of relative risk. The TDT allows accurate analysis of haplotypes, whereas computational methods to generate haplotypes from case-control studies are unreliable. Therefore, the combination of these 2 complementary approaches increases the reliability of the results collected within the study testing the association of genetic factors with susceptibility to TB.

In the case-control study, no significant association was found between alleles or genotypes of the *VDR* SNPs in any

Table 4. Genotype frequencies for single-nucleotide polymorphisms (SNPs) in the vitamin D receptor *(VDR)* in patients with severe tuberculosis (TB) and control subjects from The Gambia, Guinea, and Guinea-Bissau, combined.

SNP, genotype	Patients with TB, no. (%)	Control subjects, no. (%)	χ^2	*df*	*P*
*Fok*I			4.457	2	.108
FF	65 (52.0)	444 (61.8)			
Ff	52 (41.6)	242 (33.7)			
ff	8 (6.4)	32 (4.5)			
Total	125	718			
*Bsm*I			0.046	2	.977
bb	65 (61.3)	387 (61.0)			
bB	34 (32.1)	208 (32.8)			
BB	7 (6.6)	39 (6.2)			
Total	106	634			
*Apa*I			0.572	2	.751
aa	12 (11.3)	76 (12.0)			
aA	53 (50.0)	292 (46.0)			
AA	41 (38.7)	266 (42.0)			
Total	106	634			
*Taq*I			1.900	2	.387
tt	12 (11.3)	50 (8.0)			
tT	37 (34.9)	253 (39.9)			
TT	57 (53.8)	331 (52.2)			
Total	106	634			

NOTE. Analysis was restricted to patients with severe TB defined as having cavitations and ⩾4 zones (see table 1, bottom left).

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Table 5. Results obtained for the transmission-disequilibrium test [33] by use of *Transmit* [34].

Marker, allele/haplotype (frequency)	Observed, no.	Expected, no.	χ^2 *(df)*	*P*
*Fok*I				
F (0.787)	605	593.4	2.790	…
f (0.213)	147	158.6		
*Bsm*I				
B (0.230)	159	151.8	1.214	…
b (0.770)	497	504.2		
*Apa*I				
A (0.665)	455	437.9	4.925	.0265
a (0.335)	203	220.1		
*Taq*I				
T (0.715)	470	471.9	0.079	…
t (0.285)	188	186.1		
*Fok*I-*Bsm*I-*Apa*I-*Taq*I				
Global[a]			22.113 (9)	.0085
FbAt (0.089)	71.5	68.1	0.564	…
fbAt (0.028)	16.4	20.4	2.305	…
FBAt (0.125)	102.9	95.6	2.128	…
fBAt (0.022)	12.8	15.6	1.657	…
FbaT (0.251)	178.5	191.2	3.173	…
fbaT (0.058)	44.7	44.5	0.002	…
FbAT (0.233)	186.6	176.9	2.117	…
fbAT (0.091)	69.9	70.0	0.002	…
FBAT (0.072)	61.9	54.8	3.621	…
*Fok*I-*Bsm*I-*Apa*I				
Global[a]			15.451 (6)	.0170
Fba (0.263)	185.6	200.2	4.114	.0425
fba (0.063)	46.8	48.1	0.112	…
FbA (0.324)	259.4	246.3	3.125	…
fbA (0.118)	85.3	89.7	0.712	…
FBA (0.197)	164.3	150.4	5.260	.0218
fBA (0.030)	16.3	22.2	4.910	.0267
*Fok*I-*Apa*I				
Global			12.330 (3)	.0063
Fa (0.268)	190.8	205.0	3.917	.0478
fa (0.064)	46.6	48.7	0.287	…
FA (0.518)	421.9	395.4	11.621	.0007
fA (0.150)	102.8	113.0	3.156	…
*Bsm*I-*Apa*I				
Global[a]			5.106 (3)	.1642
ba (0.330)	199.7	215.3	4.226	.0398
bA (0.441)	295.9	287.9	0.986	…
BA (0.226)	157.1	148.6	1.743	…
*Fok*I-*Bsm*I				
Global			8.500 (3)	.0367
Fb (0.585)	445.0	445.8	0.010	…
fb (0.183)	133.0	139.8	1.107	…
FB (0.202)	167.3	154.6	4.355	.0369
fB (0.030)	16.7	21.8	4.063	.0438
*Apa*I-*Taq*I				
Global			6.962 (3)	.0731
at (0.021)	8.7	12.5	3.522	…
At (0.263)	179.3	172.5	1.054	…
aT (0.315)	194.3	208.4	3.590	…
AT (0.401)	275.7	264.6	2.031	…

NOTE. Individual single-nucleotide polymorphisms and selected haplotype results are shown. Only significant ($P < .05$) or global *P* values are listed.

[a] When low frequency haplotypes are present (<2%), these have been combined.

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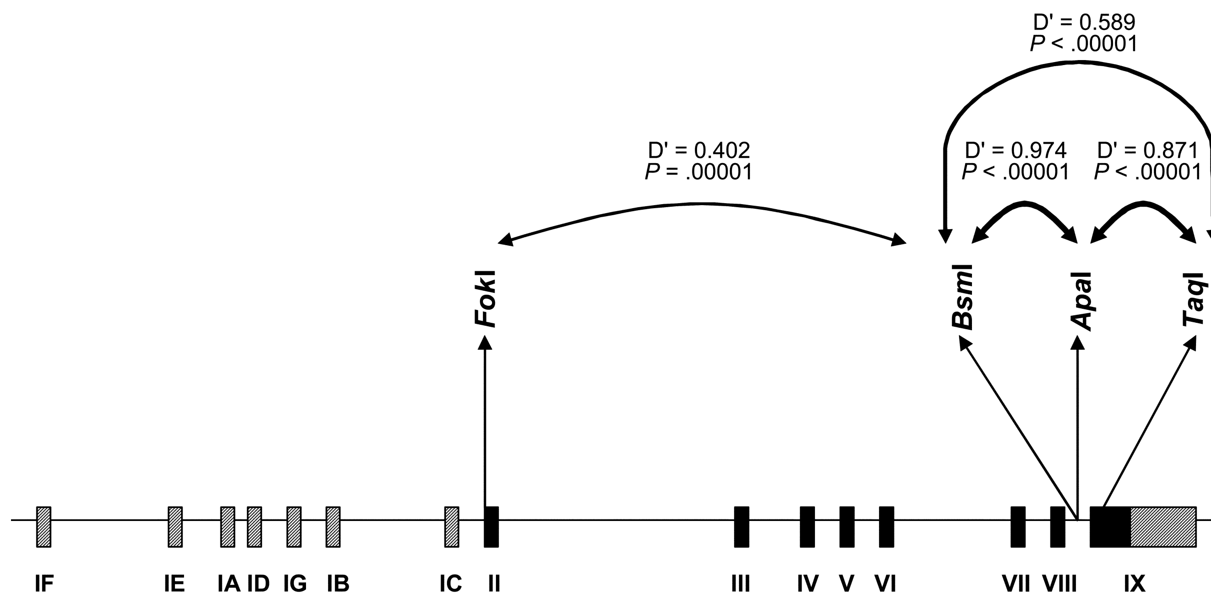

Figure 1. A schematic diagram of the vitamin D receptor *(VDR)* gene, showing approximate positions and pairwise linkage disequilibrium (LD) between polymorphisms. Maximum-likelihood estimates of pairwise LD were obtained by use of LDMAX within the GOLD software package [35]. No significant LD was detected between *Fok*I and *Apa*I ($D' = 0.117$; $P = .12$) or *Fok*I and *Taq*I ($D' = 0.141$; $P = .080$). The line weight of arrows connecting pairs of single-nucleotide polymorphisms reflects the strength in LD.

of the 3 countries or in the combined data (table 3). This observation adds to the inconsistency in the literature concerning associations found between *VDR* polymorphisms and TB (summarized in table 6). Several factors could influence the discrepancy of associations found between case-control studies of different or the same populations. First, the *VDR* is one of several polymorphic genes that have been implicated in human infectious diseases [9, 10]. Given the small effect of *VDR* on TB, the statistical power is often too low to draw conclusions about the presence or absence of an effect. Second, many of the SNPs used in association studies are nonfunctional and are more likely markers of truly causative polymorphisms. When associated alleles are truly causative, the same-risk allele would consistently be associated [27]. However, the association of nonfunctional variants depends on the patterns of LD across the relevant chromosomal region, which may differ between populations [36] and contribute to heterogeneity among associations found [27]. A high degree of genetic diversity is characteristic of African populations, giving rise to different patterns of LD and haplotype blocks [38]. Data on the worldwide distribution of *VDR* alleles and genotypes further support ethnic variation in the distribution of *VDR* variants, in particular between European and black populations [39]. This may explain the proposed ethnic-specific and unique candidate gene polymorphisms observed in certain populations [25]. Third, the interaction between different genes and/or environmental factors plays a role in the action of VDR [22, 40]. Gene-gene or gene-environment interactions most likely differ between populations. VDR is a "master" transcription factor that influences several endocrine pathways [14]. Similar to the role of *VDR* variants in bone biology [27], the association between *VDR* polymorphisms and TB is most likely confounded by numerous potential gene-gene and/or gene-environment interactions.

The significant underrepresentation of tt in case patients with TB in The Gambia reported by Bellamy et al. [13] was not confirmed by the present case-control study. In the present study, particular care was taken in the characterization and recruitment of case patients with TB and the selection of control subjects. Only those with at least 2 positive sputum smears were included in the study, and, in The Gambia, >95% of these were culture confirmed. In the study by Bellamy et al. [13], characterization of case patients with TB was less well defined, and the matching of control subjects was not as strict. Control subjects were male, whereas case patients were male and female—a potential confounder, because being female has been reported to be a protective factor against TB in Africa [6]. Differences in the power of these 2 studies, together with undetected stratification in the population sampled and variations in the way case patients with TB were characterized, could have contributed to the inconsistent observation.

Studies of the genetic components involved in disease severity after virulent *M. tuberculosis* infection have been largely limited to animals [41]. In our case-control study, we have illustrated an approach to investigate the possible involvement of *VDR* polymorphisms in TB disease severity. However, no

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Table 6. A summary of the literature verifying the association between single-nucleotide polymorphisms (SNPs) in the vitamin D receptor *(VDR)* and tuberculosis (TB).

Population	SNP(s)	Study design (no.)		Result	Reference
		Case definition	Control definition		
The Gambia	*Apa*I and *Taq*I	Smear-positive PTB (408)	Blood donor control subjects (414)[a]	tt protects ($P = .01$)	[13]
Gujarati Asians, UK	*Fok*I, *Bsm*I, and *Taq*I	Localized or severe TB (91) confirmed by biopsy or culture	Tuberculin-positive healthy contacts (116)[a]	No independent overall association	[22]
Stratified for disease phenotype		Localized (51) and severe cases (39)	Contacts (116)	f predisposes to extrapulmonary TB	
Combinations of genotype and vitamin D level		Case patients (71)	Contacts (42)	TT/Tt (non-tt) and vitamin D deficiency predisposes ($P = .017$); ff or deficient serum vitamin D levels predisposes ($P = .007$); ff or undetectable serum vitamin D levels predisposes ($P = .015$)	
Tamil-speaking south Indians	*Taq*I	Active (smear-or culture-positive) or inactive PTB (202)	Control subjects (109)[a]	No association	[23]
		Female patients (47)	Female contacts (33)	TT protects ($P < .02$); tt predisposes ($P < .02$)	
Tamil-speaking south Indians	*Taq*I	Active (smear- or culture-positive) or inactive PTB (44)	Normal healthy control subjects (32) and contacts (35)	Higher lymphocyte response to *M. tuberculosis* antigen in tt control subjects (4) ($P < .02$) and tt contacts (3); $P < .05$, vs. tt case patients (8); tuberculin reactivity in female Tt (20) > male Tt (43) ($P < .001$)	[24]
Cambodia	*Fok*I and *Taq*I	Smear-positive PTB (358)	Tuberculin-positive control subjects (106)[b]	No association	[25]
Chinese Han	*Fok*I	PTB (76)	Control subjects (171)	ff predisposes OR (95% CI) 3.668 (1.483–9.071)	[26]

NOTE. CI, confidence interval; OR, odds ratio; PTB, pulmonary TB.

[a] Control subjects were not matched for sex.

[b] Control subjects were matched for sex.

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significant association was observed between *VDR* polymorphisms and severe TB (table 4).

Family-based association tests such as the TDT are robust to population stratification but have rarely been used in studies of *VDR* polymorphisms. In the present study, family-based analysis showed that the *Apa*I A allele, alone and, more so in haplotype, with *Fok*I F (FA), was most significantly associated with TB, driving the significant global associations observed for *Fok*I-*Apa*I and possibly *Fok*I-*Bsm*I-*Apa*I-*Taq*I SNP combinations (table 5). A similar, significant global association between TB and the *Fok*I-*Bsm*I-*Apa*I-*Taq*I SNP combination was observed in a small family-based association study of the Venda population of South Africa (authors' unpublished data). The Hardy-Weinberg disequilibrium of *Apa*I in parents may influence the significance of its observed association; however, considering that the frequency of the *Apa*I AA genotype in the case patients with TB from the family study (159/329 [48.3%]) was higher than that in the control subjects from the case-control study (266/634 [42.0%]) and that the haplotype associations are so strong, this disequilibrium is unlikely to fully explain the associations reported. Variation between results obtained for the case-control and family studies may also have been influenced by the factors described above for discrepancies between different case-control studies. In addition, the mean age of case patients in the family study was 29.1 years (SD, 10.6 years), compared with 34.7 years (SD, 12.6 years) in the case-control study, which may have further contributed to the inconsistency between results.

The *Apa*I SNP has no known functional significance, and its association with TB most likely involves a nearby causative polymorphism. The A allele frequency is, on average, 29% among Asians, 53% among whites, and 67% among African Americans [39]. This higher frequency of "susceptible" variants among African populations may contribute to their increased susceptibility to TB. The more significant association found for the haplotype FA ($\chi^2 = 11.621$, $P = .0007$, 1 *df*), compared with A alone ($\chi^2 = 4.925$, $P = .0265$, 1 *df*), suggests the presence of an as-yet-untyped polymorphism that lies on this haplotype background and that is causative of the associations observed with this gene. The *Fok*I RFLP, although not independently associated with TB, has functional consequences for the action of vitamin D; the F allele in combination with the L allele of the poly A microsatellite in exon IX (L/S) increases vitamin D–induced receptor function [28] and may account for the most significant FA association. The FA haplotype, together with unidentified, associated functional alleles, may influence the function of C- and N-terminal VDR domains. Besides forming a heterodimer with RXR on direct-repeat responsive elements, VDR possesses 2 interaction interfaces with the basal transcription factor, TFIIB—1 in the N-terminal DNA-binding region and the other in the C-terminal vitamin D–binding domain [42]. The 2 biallelic variants of *Fok*I SNP in exon 2 vary in protein sequence (f/M1 being 3 aa longer than F/M4) and influence this VDR-TFIIB interaction [42]. The F/M4 protein represents a more transcriptionally active VDR isoform and is 1.5–2.5-fold more transcriptionally active than the f/M1 protein. It has been proposed that, in the tertiary structures of full-length VDR, the N- and C-terminal interaction regions combine to form a single docking scaffold for TFIIB [42]. This ensures the efficient delivery of TFIIB to the transcriptional initiation complex and transcriptional activation of vitamin D–controlled genes through the VDRE present within their promoter region. If the 3′ region of *VDR* houses functional yet unidentified polymorphisms, which are in LD with *Bsm*I, *Apa*I, and *Taq*I and encode an altered ligand-binding and heterodimerization domain, it could explain the significant association observed with the FA haplotype and 3′ polymorphisms reported here and in previous studies. The association of TB with the FA haplotype or previously described 3′ polymorphisms may be masked by environmental factors, such as the intake and light activation of vitamin D, making genotypic effects most apparent under conditions of vitamin D deficiency [22, 40, 43].

All SNPs were in LD except for *Fok*I-*Apa*I and *Fok*I-*Taq*I (figure 1). Although reports on the LD between *Fok*I and the 3′ end are limited, the results that we obtained are in general agreement with reported LD patterns. LD among markers in the 3′ region in the present study is comparable with the strength of LD observed for African Americans, departing from the almost complete LD observed for whites [36, 39].

The present study supports the proposed role for *VDR* haplotypes in susceptibility to TB, which suggests that previously associated nonfunctional alleles are most likely markers for as-yet-unidentified disease susceptibility and resistance loci. An important aim in future research would be to identify more functional sequence variants in *VDR,* to define haplotype patterns in different populations, and to understand the functional consequences of haplotypes and their interaction with environmental factors that cause differential susceptibility to TB.

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