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Association of *TNF*, *MBL*, and *VDR* Polymorphisms with Leprosy Phenotypes

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Abstract

Background—Although genetic variants in tumor necrosis factor (*TNF*), mannose binding lectin (*MBL*), and the vitamin D receptor (*VDR*) have been associated with leprosy clinical outcomes these findings have not been extensively validated.

Methods—We used a case-control study design with 933 patients in Nepal, which included 240 patients with type I reversal reaction (RR), and 124 patients with erythema nodosum leprosum (ENL) reactions. We compared genotype frequencies in 933 cases and 101 controls of 7 polymorphisms, including a promoter region variant in *TNF* (G–308A), three polymorphisms in *MBL* (C154T, G161A and G170A), and three variants in *VDR* (*FokI*, *BsmI*, and *TaqI*).

Results—We observed an association between *TNF* –308A and protection from leprosy with an odds ratio (OR) of 0.52 (95% confidence interval (CI) of 0.29 to 0.95, *P* = 0.016). *MBL* polymorphism G161A was associated with protection from lepromatous leprosy (OR (95% CI) = 0.33 (0.12–0.85), *P* = 0.010). *VDR* polymorphisms were not associated with leprosy phenotypes.

Conclusion—These results confirm previous findings of an association of *TNF* –308A with protection from leprosy and *MBL* polymorphisms with protection from lepromatous leprosy. The statistical significance was modest and will require further study for conclusive validation.

Keywords

Mycobacterium leprae; *TNF*; Mannose binding lectin; Vitamin D Receptor; Genetic polymorphism

Introduction

Leprosy, a chronic and debilitating disease caused by *Mycobacterium leprae* (ML), had a global prevalence of 213,036 in 2008 and accounted for a total of 4708 new cases from Nepal [1]. Leprosy is characterized by a spectrum of clinical manifestations from tuberculoid to lepromatous poles that correlate with the type of cell-mediated immunity that the host develops

against the bacillus [2,3]. The tuberculoid pole of leprosy (defined as polar tuberculoid (TT) or borderline tuberculoid (BT)) features a Th1 cytokine response, vigorous T cell responses to ML antigen, and containment of the infection in well-formed granulomas. At the opposite pole, lepromatous leprosy (defined as polar lepromatous (LL) or borderline lepromatous (BL)) is characterized by a Th2 immune response and poor containment of the bacillus. Two types of reactions are frequently observed in leprosy patients. Type 1 or reversal reactions (RR) represent the sudden activation of a Th1 inflammatory response to ML antigens. RR often occurs after the initiation of treatment in patients at the borderline or towards the lepromatous pole of the leprosy spectrum (LL, BL, BT or borderline borderline (BB) categories) and reflects a switch from a Th2-predominant cytokine response toward a Th1-predominant response [2, 3]. Risk factors for RR intrinsic to the host include age [4] and some genetic variants, although the latter have not been intensively investigated. Recently, we identified polymorphisms in *TLR2* (Toll-like Receptor 2), *TLR1*, and *NOD2* (Nucleotide-binding oligomerization domain) that are associated with susceptibility to RR [5–7]. Type 2 reaction or erythema nodosum leprosum (ENL) is an acute inflammatory condition involving TNF, tissue infiltration by CD4 cells [8], and deposition of immune complexes and complement [2]. ENL occurs in LL or BL patients and is more commonly seen in patients with a high bacterial index (multibacillary disease). The host factors that regulate the immunoclinical phenotypes of ENL and RR are poorly understood.

Several lines of evidence, including twin studies, genome-wide linkage studies, and candidate gene association studies, indicate that host genetic factors are important in determining susceptibility to Mycobacteria [9–11]. Studies of leprosy infection in twins have shown a three-fold greater concordance for type of leprosy disease in monozygotic compared to dizygotic twins [12]. Genome-wide linkage studies have identified two single nucleotide polymorphisms (SNPs) in the shared promoter region of the *PARK2* and the *PACRG* gene, several HLA-DR2 alleles, and a non-HLA region near chromosome 10p13 that are associated with leprosy or leprosy subtypes [10,13–15]. A recent genome-wide association study identified six genes, including *NOD2*, that were associated with leprosy susceptibility [16]. Recent studies of the innate immune response to *M. leprae* have provided hypotheses for candidate gene association studies [17]. Several receptors mediate recognition of Mycobacteria including *TLRs* 1,2,4,6,8,9, *NOD2*, *DC-SIGN*, and the mannose receptor. Genetic studies of several of these genes, as well as other immune molecules, have shown associations between leprosy phenotypes and polymorphisms, including *TLR1*, *TLR2*, *TLR4* [11], lymphotoxin- α (*LTA*) [18], the vitamin D receptor (*VDR*) [19], *TNF* (previously called *TNF- α*) [20–23], mannose binding lectin (*MBL*) [24], *NOD2* [7], and the mannose receptor [25]. Despite these suggested associations, most findings have not been replicated in independent cohorts.

TNF is a critical component of the innate and adaptive immune response and is important in Mycobacterial infection [26]. A *TNF* promoter polymorphism, G–308A, has been studied extensively [26] and have also reported an association with leprosy [20–23]. However, functional studies of the SNP –308 have demonstrated mixed results regarding its association with altered TNF levels [27,28]. MBL, is a soluble serum protein with innate immune, complement-activating, and opsonizing effects. MBL binds to carbohydrate motifs on numerous pathogens, allowing complement-mediated lysis and pathogen clearance of extracellular organisms [29]. MBL also binds lipoarabinomannan (LAM) on mycobacteria [30]. Three polymorphism in codons 52 [31], 54 [32], and 57 [33] of the first exon of the MBL gene have been studied frequently and are associated with reduced serum concentrations of MBL [29]. In a Brazilian study, haplotypes associated with increased serum concentrations of MBL were more frequent in patients with leprosy compared to controls as well as in tuberculoid compared to lepromatous patients. Vitamin D has important immunomodulatory roles, such as inhibiting DC expression of MHC II, CD40, CD80 and CD86, blocking the induction of Th1 T cell responses, and possibly promoting T regulatory cell responses [34]. Several

polymorphisms located near the 3' UTR of the VDR gene (*BsmI*, *ApaI*, and *TaqI*) are related to the stability or transcriptional activity of VDR mRNA [35], while a polymorphism located in the translation initiation codon (*FokI*) gives rise to a three amino acid difference in the VDR length that affects protein function [36]. The *TaqI* polymorphism was associated with clinical subtypes of leprosy in one study [19]. Although these studies suggest associations of these genetic variants with leprosy susceptibility, the *VDR* and *MBL* findings have not been replicated independently in separate cohorts. To our knowledge, none of the previous studies have examined associations between *TNF*, *MBL*, or *VDR* polymorphisms and leprosy reactions such as RR and ENL. In the current study, we investigated associations of these polymorphisms with leprosy, leprosy clinical subtypes and leprosy reactions.

Methods

Human Subjects and Study Design

A detailed description of study subjects and analytic methods has been published [7]. A diagnosis of leprosy and determination of leprosy type was made by clinical symptoms, skin smears and biopsy reports. Assignment of leprosy category followed the Ridley/Jopling classification scheme [37]. We enrolled 933 leprosy patients referred for treatment at Anandaban Hospital in Katmandu, Nepal and later recruited to a genetic study. Among these, 581 had lepromatous leprosy (including polar lepromatous (LL), borderline lepromatous (BL) or borderline borderline (BB)), 343 had tuberculoid leprosy (including borderline tuberculoid (BT) and polar tuberculoid (TT)), and 9 had an indeterminate classification (8 of these subjects had peripheral neuropathy). These cases comprised more than 8 different ethnic and religious groups included Brahmin (30.3%), Chetri (26.4%), Tamang (17.0%), Newar (8.6%), Magar (6.4%), Muslim (3.9%), Sarki (4.2%), and Kami (3.2%). The leprosy cases had a mean age of 44.2 with 69.9% male and 30.1% female [7]. An additional 101 unrelated controls were recruited from the same ethnic population and geographic region of Nepal. Controls were healthy individuals who had never had tuberculosis, had no history of leprosy in the family, and were living in a leprosy-endemic area. The ethnic composition of controls was Brahmin (19.1%), Chetri (31.5%), Tamang (18.0%), Newar (20.5%), Magar (3.4%), Muslim (2.3%), Sarki (2.3%), and Kami (1.1%). The controls had a mean age of 31.9 with 62.4% male and 37.6% female [7]. During 3 years of regular clinic visits, 366 patients experienced leprosy reactions, of whom 240 had RR and 128 had ENL and 2 had both reactions. Written informed consent was obtained from all participants or from their relatives if the subject could not provide consent. The study protocols were approved by the Nepal Health Research Council, the University of Washington, the University of Medicine and Dentistry of New Jersey, and the Western Institutional Review Board. The study was conducted in accord with guidelines of the US Department of Health and Human Services.

Genomic Techniques

DNA samples from the study subjects in Nepal were obtained by extraction from whole blood using Nucleon BACC2 Genomic DNA (Amersham Lifesciences) and Roche High-Pure PCR template preparation extraction kits (Roche, Germany). Genotyping was carried out with a MassARRAY technique (Sequenom) as previously described [7,38]. The following polymorphisms were genotyped: one located at promoter region of the *TNF* gene on chromosome 6p21: *TNF_G*-308A (rs1800629: G>A); three SNPs at exon 1 within a 16bp sequence in *MBL* gene located on chromosome 10q21: *MBL_C*154T for codon 52 (D-allele, rs5030737: C>T), *MBL_G*161A for codon 54 (B-allele, rs1800450: G>A) and *MBL_G*170A for codon 57 (C-allele, rs1800451: G>A); and three SNPs in the *VDR* gene located on chromosome 12q13: *VDR_FokI* (rs2228570 (previously rs10735810): T>C) in the first translation initiation codon, *VDR_BsmI* (rs1544410: G>A) in an intronic region, and *VDR_TaqI* (rs731236: T>C) in an intronic region near the 3' end. Although annotation of

VDR_FokI genotyping data in dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>) suggests that it may be multi-allelic, it appears to be bi-allelic within each population reported, including Nepal where we only observed T or C alleles. *VDR_TaqI* is a synonymous SNP and is reported to be in high LD with neighboring polymorphisms, including *BsmI* and *ApaI* [19,35]. The genotype frequencies in the control group did not deviate significantly from Hardy-Weinberg equilibrium using a Chi square (χ^2) test with $P < 0.001$ as cutoff for the level of significance.

Statistics

We evaluated the associations of polymorphisms with leprosy clinical phenotypes with allelic, genotypic, recessive and dominant models. The Chi-Square test (χ^2) was used to compare the frequency distribution in case and control groups. For the recessive model, we compared AA/Aa frequencies with the minor homozygous genotype (aa). For the dominant model, we compare genotype AA with Aa/aa frequencies. For sample sizes less than 5, a Fisher's exact test was employed. For all comparisons, the unadjusted odds ratios were calculated with 95% confidence intervals and a two-tailed test was used to evaluate statistical significance. A p value (p) of ≤ 0.05 was considered as statistically significant. Statistics were calculated with Stata software. Polymorphism frequencies were compared among several different groups. For overall leprosy susceptibility, individuals with leprosy were compared to those without leprosy. For susceptibility to different leprosy types, we compared tuberculoid (TT and BT) with lepromatous subjects (LL, BL, and BB). For the analysis of reactions, we selected control groups at risk for developing reaction. For ENL, we compared those with and without ENL in the group of leprosy patients with LL or BL. For reversal reaction, we performed 2 analyses. We compared those with and without RR within the borderline spectrum (BB, BT and BL) since that group has the highest risk of developing RR. We also compared those with and without RR within the entire leprosy case group (LL, BL, BB, BT, TT) since TT and LL individuals can develop RR at lower frequencies [4,39] (4.7% of TT and 6.0% of LL patients developed reversal reaction in our population). Due to the low number of controls without leprosy, we calculated the power ($1 - \beta$) to detect an association with 933 cases and 101 controls (assuming $\alpha = 0.05$ and $D' = 1$). In general, there was adequate power (> 0.80) to detect an odds ratio ≥ 2 for polymorphisms present at a frequency ≥ 0.1 . For polymorphisms at ≤ 0.05 frequency or for odds ratios of ≤ 1.5 , the power was not adequate (< 0.80) for detecting associations. We also evaluated the association of haplotypes with leprosy phenotypes. Haplotypes were constructed using the Expectation/Maximization (EM) algorithm in the HAPIPF program in IC Stata (version 11.0). Multivariate logistic regression was performed with Stata and adjusted for age, sex, and ethnicity when appropriate. When adjusting for ethnicity, all of the ethnic groups were included except for the unassigned ethnicity. The Chi-Square test showed non-significant changes in ethnicity between the ENL versus no ENL RR versus no RR groups. The ethnic frequencies however were significantly different between the leprosy and controls, $P < 0.01$ (data not shown).

Results

We first examined whether seven polymorphisms in *TNF*, *MBL*, and *VDR* were associated with susceptibility to leprosy by comparing the allele and genotype frequencies in the case and control groups (Table 1). Polymorphism *TNF_G*-308A was associated with protection from leprosy when comparing allele frequencies with an odds ratio of 0.52 (95% CI 0.29 – 0.95, $P = 0.016$). The genotype frequencies of this SNP also significantly differed in the leprosy cases when compared to controls ($P = 0.029$) (Table 1). With a dominant analytic model (comparing genotype AA vs Aa/aa), this variant was also associated with protection from leprosy (Table 2, OR (95% CI) = 0.55 (0.29 – 1.07), $P = 0.045$). To account for population admixture, we adjusted the analysis for ethnicity, sex, and age by multivariate logistic regression and found that the dominant analysis remained significant (OR (95% CI) = 0.46 (0.23–0.90), $P = 0.023$).

None of the other SNPs were associated with altered susceptibility to leprosy. Together, these results suggest that *TNF*_G-308A is associated with protection against leprosy.

We next examined whether these 7 polymorphisms were associated with clinical subtypes of leprosy by comparing frequencies of the tuberculoid (TT+BT) and lepromatous forms (BB+BL+LL). Variant *MBL*_G161A was associated with protection from lepromatous leprosy when comparing genotype frequencies ($P=0.030$, Table 1). This association was strongest when comparing frequencies with a recessive model (comparing AA/Aa with aa genotypes) (OR (95% CI) = 0.33 (0.12–0.85), $P = 0.010$). We next adjusted the *MBL*_G161A recessive model for ethnicity, sex and age and found that the analysis remained significant (OR (95% CI) = 0.33 (0.12–0.98), $P = 0.029$). Another polymorphism, C154T, had trends towards associations with clinical forms of leprosy in allelic and genotypic analyses that were not statistically significant (allelic comparison, OR (95% CI) = 1.75 (0.95–3.41), $P = 0.062$). When the three *MBL* polymorphisms were examined as haplotypes, no significant associations were observed except for the CAA haplotype, which was present at very low frequencies (OR (95% CI) = 0.12 (0.01–1.02), $P = 0.020$) (Table 4). None of the other SNPs were associated with leprosy type. Together, these results suggest that *MBL*_G161A is associated with altered susceptibility to clinical forms of leprosy and that there was no additive or synergistic effect of *MBL* alleles when co-inherited as haplotypes.

We next investigated whether these candidate SNPs were associated with leprosy reactions. No associations were observed between these polymorphisms and ENL when individuals within the lepromatous spectrum were analyzed. *VDR*_FokI_T (commonly known as f) allele was significantly associated with a risk of developing reversal reaction (Table 3) when individuals within the borderline spectrum (BB, BT and BL) were examined in an allelic model (OR (95% CI) = 1.31 (1.01 – 1.68), $P = 0.032$, Table 3). The allele frequency of f allele was 36.1% in those with RR versus 30.2% in those without RR. The association had borderline significance with a dominant genotypic model (OR (95% CI) = 1.39 (1.00 – 1.93), $P=0.053$). However, when the data was adjusted for ethnicity, sex, and age, the association was no longer significant (genotypic model for borderline spectrum group, $P=0.146$). A similar trend towards the association of risk of developing reversal reaction was also observed in an allelic model while comparing the reaction individuals against no reaction patients in the entire leprosy cases (OR (95% CI) = 1.25 (0.99 – 1.57), $P = 0.051$) (data not shown). Taken together, these results are inconclusive as to whether the *VDR*_FokI gene polymorphism is associated with a risk of developing reversal reaction in leprosy.

Discussion

The main findings of our study are an association of *TNF*_G-308A polymorphism with protection against leprosy and of polymorphism *MBL*_G161A with protection from lepromatous leprosy. The association of *TNF*_G-308A with protection from leprosy confirms the results of several previous studies [20,21,23]. In a study from India, the -308A allele was associated with susceptibility to lepromatous but not tuberculoid leprosy [22]. A study from southern Brazil [21] reported the opposite result with the -308A allele associated with protection from leprosy compared to healthy controls. In addition, -308A was also associated with protection from the tuberculoid type of leprosy (when comparing lepromatous and tuberculoid cases separately). In contrast to the studies mentioned above, Fitness et al did not find associations with leprosy susceptibility in Northern Malawi [40]. Similarly, we did not find an association of G-308A with either the tuberculoid or lepromatous form of leprosy. These disparate results may be due to differences in ethnicity of the study populations or the natural history of leprosy in diverse geographic settings. Furthermore, the results may be confounded by linkage disequilibrium with the highly polymorphic major histocompatibility complex (MHC) region on chromosome 6p21.3. Several studies have shown that this SNP and

others within the TNF gene are associated with different infectious diseases including tuberculosis in several independent studies and malaria [26,41,42]. However, a recent meta-analysis with a pooled sample size of 2,887 TB subjects indicated that *TNF* -308G/A SNP was not associated with TB [43].

MBL may enhance mycobacterial infection by facilitating opsonization and entry of extracellular organisms into the cell [44]. Genetic studies of *MBL* have identified both coding region polymorphisms (codons 52 (*MBL*_C154T) [31], 54 (*MBL*_G161A) [32], and 57 (*MBL*_G170A) [33]) and 3 separate promoter polymorphisms which influence circulating plasma levels of MBL. Frequency of both promoter and coding region polymorphisms vary widely in different populations [45], and extensive linkage disequilibrium between these SNPs has been noted allowing for the presence of distinct haplotypes in each population [45]. Low serum MBL levels are associated with protection from multibacillary leprosy [46]; and, in addition, leprosy patients had higher serum concentrations of MBL than unaffected controls [24,46]. In a Brazilian study, haplotypes associated with increased levels of MBL were associated with leprosy, and were more frequent in patients with lepromatous and borderline disease [24]. Other studies, however, have not confirmed this association [40]. In the present study, we analyzed the frequency of the coding region polymorphisms in *MBL* and have identified that a polymorphism associated with low MBL levels (homozygosity of *MBL*_G161A) was associated with a reduced risk of lepromatous leprosy when compared to tuberculoid leprosy. Our results are consistent with previous studies which found that the reduction of serum MBL levels is associated with protection from multibacillary disease [24, 46]. By comparison, the association of *MBL* variants with tuberculosis has been examined in several studies and the results have been heterogeneous in different populations [9].

In our study, we were not able to confirm the previously reported association of *VDR*_TaqI with specific subtypes of leprosy. The *TaqI* polymorphism of *VDR* has previously been associated with susceptibility to leprosy or tuberculoid leprosy in some studies [19,40], but not others [47]. These negative findings could be due to differences in ethnic background of the study population, sample size, other aspects of study design, or to altered virulence of *M. leprae* in different geographical locations. Although genetic variation of *M. leprae* is unusually low compared to other organisms, recent studies indicate polymorphisms and VNTRs with a strong geographical association, including strains from Nepal [48] [49]. The biologic significance of these polymorphisms and their association with different clinical phenotypes is not currently known. Moreover, the polymorphisms selected for genotyping have also not been identical in each cohort and the 3' end of the *VDR* gene contains several closely linked polymorphisms that display ethnic differences in terms of linkage [50]. It is also possible that the effect of *TaqI* polymorphism might be attributable not to the *TaqI* itself, but rather to closely linked loci (including *Apa1* or *BsmI*), that contribute variably to disease phenotype across populations.

Our study has several strengths and weaknesses. Limitations include a low number of healthy controls, which will increase the risk of Type I error. However, based on our power calculation, we should be able to identify modest associations between individual SNPs and leprosy phenotypes. Another potential limitation is the issue of multiple comparisons. If we considered a strict Bonferroni correction and multiplied the P values by seven for the number of analyzed SNPs, none of the association would survive in corrected threshold of significance. However, these SNPs were selected for their previously reported association with leprosy susceptibility and do not require the same criteria of adjustment for multiple comparison. Strengths of our study include its size and the recruitment of healthy controls from the same endemic population with comparable ethnic composition as the cases. In addition, 3 years of clinical observation enabled us to accurately determine whether subjects developed ENL or reversal reaction.

In summary, we have found associations of *TNF* and *MBL* polymorphisms with clinical outcome of leprosy and leprosy subtype in a Nepalese population. Our study replicates some of the previous findings with *TNF* with protection from leprosy and *MBL* polymorphisms with protection from lepromatous leprosy.

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Abbreviations

<i>TNF</i>	Tumor Necrosis Factor
<i>MBL</i>	Mannose Binding Lectin
<i>VDR</i>	Vitamin D Receptor
RR	Reversal Reaction
ENL	Erythema Nodosum Leprosum
TT	Tuberculoid
BT	Borderline Tuberculoid
BB	Borderline Borderline
BL	Borderline Lepromatous
LL	Lepromatous
SNPs	single nucleotide polymorphisms
HWE	Hardy-Weinberg Equilibrium

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Table 1
Association of Allele and Genotype Frequencies of *TNF*, *MBL* and *VDR* Polymorphisms with Leprosy and Leprosy type

SNP	Outcome	Allele frequency (%)		OR (95% CI)	χ^2	<i>I</i> _P	Genotype frequency (%)			χ^2	2P	HWE P
		A	a				AA	Aa	aa			
<i>TNF</i> _G-308A	Control	171 (91.0)	17 (9.0)				79 (84.0)	13 (13.8)	2 (2.1)			0.123
	Leprosy	1560 (95.1)	80 (4.9)	0.52 (0.29–0.95)	5.82	0.016	743 (90.6)	74 (9.0)	3 (0.4)	NA*	0.029 ***	
<i>MBL</i> _C154T	Control	190 (96.0)	8 (4.0)				91 (91.9)	8 (8.1)	0 (0.0)			0.675
	Leprosy	1720 (96.6)	60 (3.4)	0.83 (0.39–2.04)	0.24	0.624	831 (93.4)	58 (6.5)	1 (0.1)	NA*	0.573**	
<i>MBL</i> _G161A	Control	179 (90.4)	19 (9.6)				82 (82.8)	15 (15.2)	2 (2.0)			0.207
	Leprosy	1540 (86.9)	232 (13.1)	1.42 (0.86–2.46)	1.96	0.162	676 (76.3)	188 (21.2)	22 (2.5)	NA*	0.339***	
<i>MBL</i> _G170A	Control	197 (98.5)	3 (1.5)				97 (97.0)	3 (3.0)	0 (0.0)			0.879
	Leprosy	1741 (98.3)	31 (1.7)	1.17 (0.36–6.03)	0.07	0.797	855 (96.5)	31 (3.5)	0 (0.0)	NA*	1.000**	
<i>VDR</i> _Bsm G→A	Control	144 (72.7)	54 (27.3)				54 (54.5)	36 (36.4)	9 (9.1)			0.407
	Leprosy	1256 (72.2)	484 (27.8)	1.03 (0.73–1.46)	0.03	0.871	465 (53.4)	326 (37.5)	79 (9.1)	0.05	0.976	
<i>VDR</i> _FokI C→T	Control	139 (68.8)	63 (31.2)				45 (44.6)	49 (48.5)	7 (6.9)			0.190
	Leprosy	1220 (68.1)	572 (31.9)	1.03 (0.75–1.44)	0.04	0.832	423 (47.2)	374 (41.7)	99 (11.0)	2.57	0.277	
<i>VDR</i> _Taql T→C	Control	147 (75.8)	47 (24.2)				58 (59.8)	31 (32.0)	8 (8.2)			0.202
	Leprosy	1375 (78.8)	369 (21.2)	0.84 (0.59–1.22)	0.98	0.323	548 (62.8)	279 (32.0)	45 (5.2)	1.65	0.438	
<i>TNF</i> _G-308A	Tub	599 (94.8)	33 (5.2)				285 (90.2)	29 (9.2)	2 (0.6)			
	Lep	945 (95.3)	47 (4.7)	0.90 (0.56–1.47)	0.19	0.661	450 (90.7)	45 (9.1)	1 (0.2)	NA*	0.702**	
<i>MBL</i> _C154T	Tub	637 (97.7)	15 (2.3)				311 (95.4)	15 (4.6)	0 (0.0)			
	Lep	1068 (96.0)	44 (4.0)	1.75 (0.95–3.41)	3.49	0.062	513 (92.3)	42 (7.6)	1 (0.2)	NA*	0.131***	
<i>MBL</i> _G161A	Tub	561 (85.8)	93 (14.2)				248 (75.8)	65 (19.9)	14 (4.3)			
	Lep	965 (87.6)	137 (12.4)	0.86 (0.64–1.15)	1.15	0.283	422 (76.6)	121 (22.0)	8 (1.5)	6.99	0.030	
<i>MBL</i> _G170A	Tub	639 (97.7)	15 (2.3)				312 (95.4)	15 (4.6)	0 (0.0)			
	Lep	1086 (98.5)	16 (1.5)	0.63 (0.29–1.37)	1.68	0.195	535 (97.1)	16 (2.9)	0 (0.0)	NA*	0.192**	
<i>VDR</i> _BsmI G→A	Tub	459 (71.5)	183 (28.5)				168 (52.3)	123 (38.3)	30 (9.3)			
	Lep	787 (72.7)	295 (27.3)	0.94 (0.75–1.18)	0.31	0.578	292 (54.0)	203 (37.5)	46 (8.5)	0.30	0.862	
<i>VDR</i> _FokI C→T	Tub	455 (69.4)	201 (30.6)				162 (49.4)	131 (39.9)	35 (10.7)			

SNP	Outcome	Allele frequency (%)		OR (95% CI)	χ^2	I_P	Genotype frequency (%)			χ^2	$2P$	HWEP
		A	a				AA	Aa	aa			
	Lep	752 (67.1)	368 (32.9)	1.11 (0.90–1.37)	0.93	0.334	256 (45.7)	240 (42.9)	64 (11.4)	1.12	0.571	
VDR_ TaqI T→C	Tub	506 (78.6)	138 (21.4)				199 (61.8)	108 (33.5)	15 (4.7)			
	Lep	858 (79.2)	226 (20.8)	0.97 (0.76–1.24)	0.08	0.775	344 (63.5)	170 (31.4)	28 (5.2)	0.49	0.782	

HWEP = Hardy-Weinberg Equilibrium P value, a value >0.001 indicates that polymorphism is in Hardy-Weinberg Equilibrium.
NA, Not available.

I_P P value for comparison of allele frequencies by Chi-square unless otherwise indicated. "A" denotes common allele and "a" denotes minor allele. P values <0.05

$2P$ P value for comparison of genotype frequencies calculated by Chi square unless otherwise indicated.

* Fisher exact test computes p value directly and therefore is not associated with Chi square values.

** Corrected p value by Fisher's exact due to small cell frequencies

Table 2
Association of *TNF*, *MBL* and *VDR* Polymorphism with Leprosy & Leprosy Type with Dominant and Recessive Models

SNP	Outcome	Recessive		OR	χ^2	<i>I</i> ²	Dominant		OR	χ^2	<i>I</i> ²
		AA + Aa n (%)	aa n (%)				AA n (%)	Aa + aa n (%)			
<i>TNF</i> _G-308A	Control	92 (97.9)	2 (2.1)				79 (84.0)	15 (16.0)			
	Leprosy	817 (99.6)	3 (0.4)	0.17 (0.02–2.05)	NA *	0.085 **	743 (90.6)	77 (9.4)	0.55 (0.29–1.07)	4.02	0.045
<i>MBL</i> _C154T	Control	99 (100.0)	0 (0.0)				91 (91.9)	8 (8.1)			
	Leprosy	889 (99.9)	1 (0.1)	NA	NA *	1.000 **	831 (93.4)	59 (6.6)	0.81 (0.37–2.02)	0.30	0.586
<i>MBL</i> _G161A	Control	97 (98.0)	2 (2.0)				82 (82.8)	17 (17.2)			
	Leprosy	864 (97.5)	22 (2.5)	1.23 (0.30–10.99)	NA *	1.000 **	676 (76.3)	210 (23.7)	1.50 (0.86–2.76)	2.14	0.143
<i>MBL</i> _G170A	Control	100 (100.0)	0 (0.0)				97 (97.0)	3 (3.0)			
	Leprosy	886 (100.0)	0 (0.0)	NA	NA *	0.000 **	855 (96.5)	31 (3.5)	1.17 (0.36–6.10)	0.07	1.000
<i>VDR</i> _BsmI G→A	Control	90 (90.9)	9 (9.1)				54 (54.5)	45 (45.5)			
	Leprosy	791 (90.9)	79 (9.1)	1.00 (0.48–2.34)	0.00	0.997	465 (53.4)	405 (46.6)	1.05 (0.67–1.63)	0.04	0.836
<i>VDR</i> _FokI C→T	Control	94 (93.1)	7 (6.9)				45 (44.6)	56 (55.4)			
	Leprosy	797 (89.0)	99 (11.0)	1.67 (0.75–4.38)	1.62	0.203	423 (47.2)	473 (52.8)	0.90 (0.58–1.39)	0.26	0.612
<i>VDR</i> _TaqI T→C	Control	89 (91.8)	8 (8.2)				58 (59.8)	39 (40.2)			
	Leprosy	827 (94.8)	45 (5.2)	0.61 (0.27–1.54)	1.61	0.205	548 (62.8)	324 (37.2)	0.88 (0.56–1.39)	0.35	0.556
<i>TNF</i> _G-308A	Tuberculoid	314 (99.4)	2 (0.6)				285 (90.2)	31 (9.8)			
	Lepromatous	495 (99.8)	1 (0.2)	0.32 (0.01–6.12)	0.98	0.564	450 (90.7)	46 (9.3)	0.94 (0.57–1.57)	0.06	0.799
<i>MBL</i> _C154T	Tuberculoid	326 (100.0)	0 (0.0)				311 (95.4)	15 (4.6)			
	Lepromatous	555 (99.8)	1 (0.2)	NA	NA *		513 (92.3)	43 (7.7)	1.74 (0.93–3.42)	3.28	0.070
<i>MBL</i> _G161A	Tuberculoid	313 (95.7)	14 (4.3)				248 (75.8)	79 (24.2)			
	Lepromatous	543 (98.5)	8 (1.5)	0.33 (0.12–0.85)	6.73	0.010	422 (76.6)	129 (23.4)	0.96 (0.69–1.34)	0.06	0.801
<i>MBL</i> _G170A	Tuberculoid	327 (100.0)	0 (0.0)				312 (95.4)	15 (4.6)			
	Lepromatous	551 (100.0)	0 (0.0)	NA	NA *		535 (97.1)	16 (2.9)	0.62 (0.28–1.37)	1.71	0.191
<i>VDR</i> _BsmI G→A	Tuberculoid	291 (90.7)	30 (9.3)				168 (52.3)	153 (47.7)			
	Lepromatous	495 (91.5)	46 (8.5)	0.90 (0.54–1.51)	0.18	0.673	292 (54.0)	249 (46.0)	0.94 (0.70–1.25)	0.22	0.641

SNP	Outcome	Recessive		OR	χ^2	I_P	Dominant		OR	χ^2	I_P
		AA + Aa n (%)	aa n (%)				AA n (%)	Aa + aa n (%)			
VDR_ FokI C→T	Tuberculosis	293 (89.3)	35 (10.7)				162 (49.4)	166 (50.6)			
	Lepromatous	496 (88.6)	64 (11.4)	1.08 (0.69–1.72)	0.12	0.729	256 (45.7)	304 (54.3)	1.16 (0.87–1.54)	1.12	0.290
VDR_ TaqI T→C	Tuberculosis	307 (95.3)	15 (4.7)				199 (61.8)	123 (38.2)			
	Lepromatous	514 (94.8)	28 (5.2)	1.11 (0.56–2.28)	0.11	0.740	344 (63.5)	198 (36.5)	0.93 (0.69–1.25)	0.24	0.624

N/A, not available.

I_P P value comparison by Chi-square (χ^2) unless otherwise indicated. P values <0.05 are in bold.

* Fisher exact test computes p value directly and therefore is not associated with Chi square values.

** Corrected p value by Fisher's exact due to small cell frequencies

Table 3

Association of *TNF*, *MBL* and *VDR* Polymorphisms with Leprosy Reactions

SNPs variable	Outcome	Allele frequency (%)		OR (95% CI)	χ^2	<i>I</i> _p	Genotype frequency (%)			χ^2	2p
		A	a				AA	Aa	aa		
<i>TNF</i> _G-308A	No RR	727 (94.4)	43 (5.6)				344 (89.4)	39 (10.1)	2 (0.5)		
	RR	374 (95.9)	16 (4.1)	0.72 (0.38–1.33)	1.18	0.278	180 (92.3)	14 (7.2)	1 (0.5)	1.36	0.549
<i>MBL</i> _C154T	No RR	797 (96.5)	29 (3.5)				384 (93.0)	29 (7.0)	0 (0.0)		
	RR	422 (97.7)	10 (2.3)	0.65 (0.28–1.39)	1.35	0.245	206 (95.4)	10 (4.6)	0 (0.0)	1.40	0.297
<i>MBL</i> G161A	No RR	703 (85.7)	117 (14.3)				306 (74.6)	91 (22.2)	13 (3.2)		
	RR	373 (86.3)	59 (13.7)	0.95 (0.67–1.35)	0.09	0.768	163 (75.5)	47 (21.8)	6 (2.8)	0.10	0.985
<i>MBL</i> _G170A	No RR	811 (97.9)	17 (2.1)				397 (95.9)	17 (4.1)	0 (0.0)		
	RR	418 (98.1)	8 (1.9)	0.91 (0.34–2.26)	0.04	0.833	205 (96.2)	8 (3.8)	0 (0.0)	0.05	1.000
<i>VDR</i> _BsmI G→A	No RR	587 (72.5)	223 (27.5)				216 (53.3)	155 (38.3)	34 (8.4)		
	RR	310 (74.5)	106 (25.5)	0.90 (0.68–1.19)	0.59	0.443	118 (56.7)	74 (35.6)	16 (7.7)	0.64	0.731
<i>VDR</i> _FokI C→T	No RR	581 (69.8)	251 (30.2)				209 (50.2)	163 (39.2)	44 (10.6)		
	RR	276 (63.9)	156 (36.1)	1.31 (1.01–1.68)	4.60	0.032	91 (42.1)	94 (43.5)	31 (14.4)	4.33	0.111
<i>VDR</i> _TaqI T→C	No RR	645 (79.6)	165 (20.4)				260 (64.2)	125 (30.9)	20 (4.9)		
	RR	332 (78.3)	92 (21.7)	1.08 (0.80–1.46)	0.30	0.585	128 (60.4)	76 (35.8)	8 (3.8)	1.80	0.432
<i>TNF</i> _G-308A	No ENL	677 (94.8)	37 (5.2)				321 (89.9)	35 (9.8)	1 (0.3)		
	ENL	218 (96.5)	8 (3.5)	0.67 (0.27–1.49)	1.02	0.314	105 (92.9)	8 (7.1)	0 (0.0)	NA*	0.587**
<i>MBL</i> _C154T	No ENL	790 (96.1)	32 (3.9)				380 (92.5)	30 (7.3)	1 (0.2)		
	ENL	229 (96.2)	9 (3.8)	0.97 (0.40–2.12)	0.01	0.937	110 (92.4)	9 (7.6)	0 (0.0)	NA*	1.000**
<i>MBL</i> _G161A	No ENL	710 (88.1)	96 (11.9)				313 (77.7)	84 (20.8)	6 (1.5)		
	ENL	205 (85.4)	35 (14.6)	1.26 (0.81–1.94)	1.21	0.272	86 (71.7)	33 (27.5)	1 (0.8)	NA*	0.266**
<i>MBL</i> _G170A	No ENL	802 (98.8)	10 (1.2)				396 (97.5)	10 (2.5)	0 (0.0)		
	ENL	234 (98.3)	4 (1.7)	1.37 (0.31–4.80)	0.28	0.595	115 (96.6)	4 (3.4)	0 (0.0)	NA*	0.532**
<i>VDR</i> _BsmI G→A	No ENL	574 (72.1)	222 (27.9)				212 (53.3)	150 (37.7)	36 (9.0)		
	ENL	174 (74.4)	60 (25.6)	0.89 (0.63–1.25)	0.46	0.498	66 (56.4)	42 (35.9)	9 (7.7)	0.43	0.831
<i>VDR</i> _FokI C→T	No ENL	550 (66.4)	278 (33.6)				187 (45.2)	176 (42.5)	51 (12.3)		

SNPs variable	Outcome	Allele frequency (%)		OR (95% CI)	χ^2	I_P	Genotype frequency (%)			χ^2	$2P$
		A	a				AA	Aa	aa		
	ENL	171 (71.3)	69 (28.8)	0.80 (0.57–1.10)	1.97	0.160	59 (49.2)	53 (44.2)	8 (6.7)	3.07	0.223
<i>VDR</i> <i>TaqI</i> T→C	No ENL	619 (78.0)	175 (22.0)				244 (61.5)	131 (33.0)	22 (5.5)		
	ENL	193 (81.8)	43 (18.2)	0.79 (0.53–1.15)	1.59	0.207	80 (67.8)	33 (28.0)	5 (4.2)	1.60	0.481

NA, not available.

I_P P value for comparison of allele frequencies by Chi-square (χ^2) unless otherwise indicated. “A” denotes common allele and “a” denotes minor allele. P values <0.05 are in bold. The analysis of reversal reaction (RR) compares those with and without RR within the group of leprosy cases with BB, BT or BL. The analysis of erythema nodosum leprosum (ENL) compares those with and without ENL within the group of leprosy cases with BL or LL.

$2P$ P value for comparison of genotype frequencies by Chi-square (χ^2) unless otherwise indicated.

* Fisher exact test computes p value directly and therefore is not associated with Chi square values.

** Corrected p value by Fisher's exact due to small cell frequencies.

Table 4

haplotypes with Leprosy and Leprosy Reactions

Haplotype	Yes (f)	OR	2p	Leprosy Class		OR	2p	Reversal reaction		OR	2p	ENL		OR	2p
				TT+BT (f)	BB+BL+LL (f)			No (f)	Yes (f)			No (f)	Yes (f)		
1 (0.85)	1420.1 (0.82)			525.7 (0.82)	881.0 (0.82)			1048.3 (0.82)	371.2 (0.82)			653.2 (0.83)	186.6 (0.80)		
2 (0.05)	218.8 (0.06)	1.35 (0.82–2.23)	0.235	86.9 (0.07)	130.5 (0.06)	0.90 (0.67–1.20)	0.464	161.7 (0.06)	57.8 (0.06)	1.01 (0.73–1.39)	1.009	89.8 (0.06)	34.8 (0.07)	1.36 (0.89–2.07)	0.157
3 (0.4)	56.9 (0.03)	0.85 (0.39–1.84)	0.682	14.3 (0.02)	42.0 (0.04)	1.75 (0.95–3.21)	0.069	44.7 (0.03)	12.8 (0.03)	0.81 (0.43–1.52)	0.808	29.8 (0.04)	8.4 (0.04)	0.98 (0.45–2.15)	0.967
4 (0.2)	24.1 (0.01)	0.93 (0.28–3.12)	0.906	9.5 (0.01)	14.5 (0.01)	0.91 (0.40–2.08)	0.826	18.0 (0.01)	6.0 (0.01)	0.94 (0.37–2.39)	0.943	9.0 (0.01)	3.6 (0.02)	1.39 (0.40–4.78)	0.602
5 (0)	6.0 (0.00)	NA	0.405	5.0 (0.01)	1.0 (0.00)	0.12 (0.01–1.02)	0.020	4.0 (0.00)	2.0 (0.00)	1.41 (0.26–7.74)	1.412	1.0 (0.00)	0.0 (0.00)	0.00 (0.01–61.37)	0.593

Left to right: C154T, G161A

Comparison of a given haplotype with reference to haplotype CCG. P values <0.05 are in bold