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Common polymorphisms in the *NOD2* gene region are associated with leprosy and its reactive states

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Abstract

Background—Because of the wide spectrum of clinical manifestations and well-defined immunologic complications, leprosy is a useful disease for studying genetic regulation of the host response to infection. We hypothesized that polymorphisms in *NOD2*, a cytosolic receptor known to detect mycobacteria, are associated with susceptibility to leprosy and its clinical outcomes.

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 type 2 (erythema nodosum leprosum (ENL)) reactions. We compared 32 common *NOD2* gene region polymorphism frequencies between the different clinical types of leprosy as well as 101 controls without leprosy.

Results—Four polymorphisms were associated with leprosy susceptibility when comparing allele frequencies and eight were associated when comparing genotype frequencies with a dominant model. Five polymorphisms were associated with protection from RR in an allelic analysis, and seven were associated with RR with a dominant model. Four polymorphisms were associated with increased susceptibility to ENL in an allelic analysis, while seven of 32 polymorphisms were associated with a dominant model.

Conclusion—These data suggest that *NOD2* genetic variants are associated with leprosy susceptibility and the development of leprosy reactive states.

Keywords

Mycobacterium leprae; *NOD2*; Reversal Reaction; Erythema nodosum leprosum; Genetic polymorphisms; CYLD; SLIC1

Introduction

Leprosy is an infectious disease caused by *Mycobacterium leprae* that involves the peripheral nerves and skin with a polarized clinical presentation [1-3]. At one end of the spectrum is

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tuberculoid leprosy (TT), which is characterized by isolated skin lesions with well-formed granulomas, rare intracellular bacilli on biopsy, and a Th1 dominant T cell cytokine response. At the other end of the spectrum, lepromatous leprosy (LL) patients have numerous lesions with poorly formed granulomas rich in foamy macrophages containing abundant intravacuolar bacilli, and a Th2 dominant T cell response. Between the two poles are patients with borderline lepromatous (BL), mid borderline (BB) and borderline tuberculoid (BT) disease that manifest intermediary bacillary loads and cellular immunity to *M. leprae* and its antigens. Thirty to 50 percent of leprosy patients can also develop one of two reactional states [1]. Type I or reversal reaction (RR) typically occurs in borderline forms of disease (BL or BB) and is associated with a rapid shift of T cell immunity to the tuberculoid pole (or Th1) or less often to the lepromatous (or Th2) pole, both leading to severe tissue damage. Type 2 reactions or erythema nodosum leprosum (ENL) typically occurs in patients with BL or LL leprosy, and is characterized by increased TNF-α serum levels and immune complex-associated vasculitis, panniculitis and uveitis. ENL and RR may manifest during antimicrobial treatment, but often they occur before or even years after treatment. Host factors that control clinical disease course in leprosy remain only partially understood. We hypothesize common genetic variation in innate immune receptors influence the immune response and diverse clinical phenotypes in patients with leprosy.

Many studies suggest that human genetic factors influence the acquisition of leprosy as well as the clinical course and type of disease. The first studies that suggested genetic influence demonstrated that monozygotic twins have a three-fold greater similarity in type of leprosy than dizygotic twins [4]. Linkage studies have identified PARK2 and PACRG as well as chromosomal regions 10p13 and 20q12 as leprosy susceptibility loci [5,6]. Candidate gene studies have identified polymorphisms in several immune genes associated with leprosy susceptibility including the vitamin D receptor, lymphotoxin-α, TNF-α, IL-10, HLA genes and TAP-2 [7-9]. Since the spectrum of macrophage intracellular replication can be multibacillary in lepromatous disease and paucibacillary in tuberculoid disease [10], focus has turned to gene studies in the innate immune system. To date candidate genes in this area have focused on pathogen receptors located on the surface and intracellular organelles and have identified Toll-like receptor 1 (TLR-1) [11], TLR-4 [12] and mannose binding lectin [13,14] as important in leprosy disease susceptibility. In addition, we recently identified polymorphisms in TLR-2 and TLR-1 that are associated with susceptibility to RR [15,16].

In addition to receptors such as TLRs on the cell surface and within phagosomes, the innate immune system has cytosolic receptors to detect intracellular pathogens. Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are a diverse set of 22 innate immune receptors involved in cytoplasmic detection of microbes and activation of inflammatory cascades [17,18]. The association of genetic variation in NLRs and cytosolic receptors with infectious disease susceptibility is poorly understood. Due to *M. leprae*'s ability to replicate intracellularly, we hypothesized that cytoplasmic receptors may also play an important role in pathogen detection and may be associated with the varied clinical forms of disease. Although the predominant site of *M. leprae* replication is within the vacuole, there is some evidence that egress of *Mycobacteria* [19] and/or mycobacterial cell wall lipids and other bacillary components into the cytosol may also occur [20] via a conserved secretion system (ESX-1) dependant process to activate the inflammasome [21,22]. *NOD2* (CARD15) contains leucine rich repeats linked to effector domains (Caspase activation recruitment domains (CARD)) and activates NF-κb nuclear translocation through activation of Rip2 kinase. *NOD2* detects the cell wall building block muramyl dipeptide (MDP), and plays a role in the immune response to many pathogens [23].

Human variants of *NOD2* have been detected in Crohn's disease [24,25], a chronic granulomatous disorder of the gut that appears similar to Johnne's disease, a granulomatous

disorder in cows caused by *Mycobacteria paratuberculosis*. The association of Crohn's disease with mycobacterial infection, however, remains controversial [26,27]. A number of studies have implicated *NOD2* directly in mycobacterial disease. Nonsynonomous variants of *NOD2* were more frequent in patients with active tuberculosis (TB) in a cohort of patients from Houston [28]. In addition, human and murine macrophages detect *M. tuberculosis* and *M. paratuberculosis* in a *NOD2*-dependent manner [29,30]. *NOD2* also influences TB disease course in the mouse model of infection [31,32]. The function of *NOD2*, however, in *M. leprae* infection has not been addressed. In this paper, we show evidence for allelic and genotypic association of *NOD2* genetic variants with leprosy reactive states and leprosy susceptibility.

Materials and Methods

Human subjects

Recruitment and description of the patient population was previously reported [16]. Briefly, 933 study participants were referred to Anandaban Hospital in Katmandu, Nepal for treatment of leprosy. The diagnosis of leprosy in these patients was done on the basis of clinical symptoms, skin smears, and biopsy reports. The Ridley/Jopling classification scheme was used to assign the class of leprosy. Following initiation of treatment, patients were followed for the development of reactive states (RR and ENL) for a minimum of 3 years. The institutional review boards at University of Washington, University of Medicine and Dentistry of New Jersey, Nepal Health Research Council, and Western Institutional Review Boards approved patient protocols as per the US Department of Health and Human Services Guidelines. Written informed consent was obtained from all of the patients. Eight different ethnic and religious groups were included in the study including Brahmin (26.0%), Chetri (24.2%), Tamang (14.7%), Newar (8.7%), Magar (5.2%), Sarki (3.4%), Muslim (3.3%), and Kami (2.7%), with 11.8% having unrecorded ethnicity [16].

Determination of SNP for genotypic analysis

We identified haplotype tagging SNPs from the Han Chinese (CHB) and European (CEU) ancestry from the International HapMap Project database (http://www.hapmap.org). The genomic region adjacent to *NOD2* (16q12) also contains the genes for familial Cylindromatosis (CYLD) and selectin ligand interactor cytoplasmic protein - 1 (*SLIC1* or *SNX-20*). We searched a region on chromosome 16q12, 50 kilobases upstream and downstream of genes *NOD2* and *CYLD* for tagged SNPs using an R^2 cutoff of 0.8 for linkage disequilibrium and a minor allele frequency cutoff of 10%. Also added were non-synonomous SNPs found in these two populations at frequencies of greater than 5%. Using this search technique we identified 32 unique SNPs to analyze in this region (Figure 1).

Molecular Biology

DNA was obtained from whole blood using Nucleon BACC2 Genomic DNA (Amersham Lifesciences) and High-Pure PCR template preparation extraction kits (Roche) as previously described [16]. Genotyping of DNA was performed using a chip-based MALDI-TOF MassARRAY technique (Sequenom) as previously described [16]. Briefly, SPECTRODE-SIGNER software was used to determine probes to be used for multiplex SNP assays; 5ng of DNA was amplified in a 384-well plate following Sequenom's specifications. Shrimp alkaline phosphatase was added after PCR to prevent further incorporation of unused dNTPs that might interfere with the primer extension step. Next allelic discrimination reactions were performed by adding a mixture of dNTPs and dideoxy NTPs to each well. MassEXTEND clean resin was added to the mixture to remove extraneous salts that could interfere with the MALDI-TOF analysis. Genotypes were determined by spotting 15 nl of each reaction onto a 386

SpectroCHIP (seqenom), which was subsequently read by the MALDI-TOF mass spectrometer.

Statistics

Univariate analysis was performed on categorical variables using a Pearson Chi-square test, and for continuous variables using a Student's t test. Multivariate logistic regression was performed using Stata (Intercooled Version 10.1, Statacorp) and adjusted for age groups, sex, and ethnicity when appropriate. Haplotypes were constructed with the program Hapipf in Stata. For each of the *NOD2* polymorphisms, significant deviations from the expected genotypic frequencies were determined using the Hardy Weinberg principle with a p value cutoff of $p<0.001$. All SNPs analyzed were in Hardy Weinberg equilibrium when analyzed in the control group.

Multiple Tests Comparisons

32 polymorphisms were genotyped in our study in three analyses, ENL versus unaffected, RR versus unaffected, and leprosy versus control. If we correct for multiple comparisons with a Bonferroni test (P value × 32), only p values less than 0.00156 remain significant. However, linkage disequilibrium between the 32 polymorphisms, is significant (Figure 1) and suggests that a Bonferroni correction is overly conservative [33]. Since there is no standardized approach for correcting P values for multiple comparisons in candidate gene association studies, we report uncorrected P values throughout the manuscript.

Results

Association of NOD2 SNPs with leprosy acquisition in Nepal

To determine whether NOD2 polymorphisms are associated with leprosy susceptibility, we compared allele and genotype frequencies in 933 leprosy patients and 101 controls without leprosy. Baseline characteristics of these subjects are listed in Table 1. Four polymorphisms were identified as significant ($p<0.05$) at the allelic level, (rs12448797 (OR 2.18, 95%CI 1.05-4.52, p=0.031), rs2287195 (OR 1.51, 95%CI 1.09-2.10, p=0.013), rs8044354 (OR 1.53, 95%CI 1.12-2.07, p=0.006), and rs1477176 (OR 0.44, 95%CI 0.29-0.68, p=0.0002)) (Table 2). The most significant of these was rs1477176 (OR 0.44, 95%CI 0.29-0.68, p=0.00017). When these genotypes were analyzed with a dominant model, 8 of these SNPs were associated with leprosy susceptibility (Supplemental Table 1). Six of these SNPs (rs2287185, rs8044354, rs8043770, rs13339578, rs4785225, and rs751271) were clustered in the promoter region and within the *NOD2* gene itself. The remaining two SNPs (rs12448797 and rs1477176) were located in adjacent genes (selectin ligand interactor cytoplasmic protein (*SLIC1*) and ubiquitin carboxyl-terminal hydrolase (*CYLD* - cylindromatosis)).

We next examined whether population admixture could confound our results as some differences were seen in the ethnic frequencies between control individuals and the leprosy patients. There are greater than eight ethnic groups in Nepal, and the majority (approximately 70%) are present in 4 groups (Brahmin, Chetri, Tamang and Newar). We used a multivariate logistic regression model to adjust the analysis for ethnicity (with the 8 prevalent ethnic groups, excluding uncategorized), age and sex when comparing genotype frequencies in a dominant model. When adjusted for ethnicity, sex, and age, eight out of eight polymorphisms (rs12448797 (OR 3.20, 95%CI 1.24-8.26, p=0.016), rs2287195 (OR 2.29, 95%CI 1.43-3.68, p=0.001), rs8044354 (OR 2.17, 95%CI 1.36-3.46, p=0.001), rs8043770 (OR 2.05, 95%CI 1.25-3.34, p=0.004), rs13339578 (OR 2.19, 95%CI 1.37-3.52, p=0.001), rs4785225 (OR 2.00, 95%CI 1.25-3.21, p=0.004), rs751271 (OR 1.95, 95%CI 1.22-3.13, p=0.005), and rs1477176 (OR 0.43, 95%CI 0.25-0.76, p=0.003)) remained significantly associated with leprosy

susceptibility. Together, these results suggest that several *NOD2* SNPs are associated with leprosy susceptibility.

Lack of association of NOD2 with Leprosy Type

Of the 933 patients enrolled in the study, 582 had lepromatous leprosy (including LL, BL, or BB), and 342 had tuberculoid leprosy (including BT and TT leprosy). We analyzed the frequency of *NOD2* SNPs to associate with type of leprosy. Only one SNP (rs1131716) was associated with predisposition to the lepromatous pole (OR 2.01, 95%CI 1.12-3.76, p=0.013). All other SNPs did not show association with leprosy type (data not shown).

Association of NOD2 SNPs with RR in leprosy

Among 933 leprosy subjects in Nepal, 240 individuals had type 1 RR over a three-year period of regular visits to the leprosy clinic. Of these, only 2 were identified as also having ENL.

We compared the minor allele frequency of those with and without RR and identified five SNPs (Table 3), (rs2287195 Odds Ratio (OR) 1.34, p=0.013, rs8044354 OR 1.36, p=0.005, rs8043770, OR 1.36, p=0.012, rs7194886 OR 1.36, p=0.032, and rs1861759, OR 1.33, p=0.041) with significant association. We also found one SNP with borderline significance (rs4785225, OR 1.25, p=0.064) (Table 3). The most significant association was with SNP rs8044354 (OR 1.36 (95%CI 1.09-1.70), p=0.005). The polymorphisms were clustered in the gene regions located between the *NOD2* and *SLIC1* genes (Figure 1).

We next analyzed the distribution of genotype frequencies (Table 3). We found 3 polymorphisms with significantly different genotype frequencies (rs2287195, rs8044354, and rs8043770) in those with RR versus no RR (Table 2). Next, we examined whether these genotypic associations were consistent with a recessive (00 + 01 vs 11) or dominant (00 vs 01 + 11) model. We found that seven *NOD2* polymorphisms had significant associations (P value <0.05) with protection from RR assuming a dominant inheritance model (Supplemental Table 1). Of these 7 SNPs, two polymorphisms (rs4785225 and rs752171) were noted to be in significant linkage disequilibrium with an R^2 value of greater than 0.8 in this population.

We next evaluated whether the associations were affected by population admixture. No significant difference in ethnicity (p=0.319) or sex (p=0.710) was observed between people affected with RR and those not affected (Table 1). Those affected with RR tended to be significantly younger at the time of sample collection (36.7 years old versus 45.9 years old) than those unaffected (p<0.01) (Table 1). In an adjusted analysis (for age, sex, and ethnicity), SNP rs2287195 (OR 0.70, 95%CI 0.49-1.00, p=0.049), rs8043770 (OR 0.68, 95%CI 0.48-0.96, p=0.028), rs7194886 (OR 0.63, 95%CI 0.44-0.92, p=0.018), and rs1861759 (OR 0.66, 95%CI 0.47-0.95, p=0.027) remained associated with protection from RR. In contrast, three other SNPs (rs8044354, rs4785225, and rs751271) when adjusted for age, sex and ethnicity did not remain associated with RR. Together, these results suggest that several SNPs in *NOD2* may be associated with protection from RR.

Association of NOD2 SNPs with ENL in leprosy

We next examined whether polymorphisms in the *NOD2* gene region were associated with erythema nodosum leprosum (ENL) reaction in leprosy. A total of 124 patients identified with ENL were compared to 428 patients with lepromatous or borderline lepromatous leprosy (LL or BL) without ENL (Table 1). No significant differences in ethnicity were seen between leprosy patients who were affected with ENL versus those who were not (p=0.578). At the allelic level there were 4 SNPs which had significant associations (rs8044354 (OR 0.74, p=0.046), rs17312836, (OR 0.70, p=0.039), rs1861759 (OR 0.70, p=0.037), and rs1861758

(OR 0.71, p=0.047)) with p values less then 0.05 (Table 4), and 2 SNPs with borderline significance (rs6500328 (OR 0.73, p=0.066), and rs7194886 (OR 0.72, p=0.057)).

Genotypic analysis revealed that these associations with ENL were significant for seven of the SNPs (rs2287195, rs8044354, rs7194886, rs6500328, rs17312836, rs1861759, and rs1861758) (Table 4). We also found that 7 polymorphisms were associated with susceptibility in a dominant model (Supplemental Table 1). The most significant was SNP rs8044354 (OR 2.17 (95%CI 1.26-3.88), p=0.003). Four of these 7 polymorphisms were in linkage disequilibrium in one haplotype block (one group of 4 SNPs (rs6500328, rs17312836, rs1861759, and rs1861758)). When adjusted for ethnicity, sex, and age seven out of seven polymorphisms (rs2287195 (OR 1.93, 95%CI 1.14-3.30, p=0.015), rs8044354 (OR 2.83, 95%CI 1.52-5.28, p=0.001), rs7194886 (OR 1.73, 95%CI 1.07-2.82, p=0.027), rs6500328 (OR 1.90, 95%CI 1.18-3.07, p=0.008), rs17312836 (OR 1.97, 95%CI 1.22-3.19, p=0.005), rs1861759 (OR 2.00, 95%CI 1.25-3.22, p=0.004), and rs1861758 (OR 2.05, 95%CI 1.26-3.33, p=0.004)) remained significantly associated with ENL. Together, these data suggest that several *NOD2* SNPs are associated with ENL susceptibility.

NOD2 haplotypes are associated with ENL and RR

We next constructed haplotypes of the polymorphisms that were significantly associated with RR or ENL. Ten of the 32 polymorphisms were included in the analysis (rs2287195 (0=A, 1=G), rs8044354 (0=A,1=G), rs8043770 (0=C,1=G), rs7194886 (0=C,1=T), rs6500328 (0=A, 1=G), rs17312836 (0=C,1=G), rs1861759 (0=A,1=C), rs4785225 (0=C,1=G), rs751271 (0=A, 1=C), and rs1861758 (0=C,1=T)). We compared whether the haplotype frequencies were significantly different in those who were affected by leprosy reactional states (ENL or RR). For ENL we found one haplotype (1.1.1.1.1.1.1.1.1.1, OR 1.60, 95%CI 1.03-2.48, p=0.035) which conferred significant susceptibility to development of ENL reaction. For RR we found 1 haplotype with significant differences that conferred significant protection to RR (1.1.1.1.1.1.1.1.1.1, OR 0.647, 95%CI 0.45-0.93, p=0.020). These results suggested that haplotype analyses did not reveal greater associations than examination of single polymorphisms.

Together these results indicate that variation in the *NOD2* gene region is associated with increased susceptibility to ENL, and conversely are associated with protection from Type 1 RR in leprosy. No association was observed when comparing polar leprosy types (lepromatous versus tuberculoid).

Discussion

In the present study, we used a candidate gene approach to identify an association of the chromosome 16q12 loci with leprosy occurrence and the development of leprosy reactional states in a Nepalese population. We identify several *NOD2* polymorphisms that are associated with leprosy susceptibility in the Nepalese population, identifying this gene as an important factor in protection against intracellular pathogens. We also found several polymorphisms are either associated with protection from RR or are associated with increased susceptibility to ENL. To our knowledge, this study is the first to associate variation in the *NOD2* region with leprosy reactional states and the first to identify genetic associations with ENL; *NOD2* is the third signaling receptor gene to be associated with RR in leprosy; the other two, TLR2 and TLR1, are important in the extracellular detection of organisms [16,34].

Like many gene association studies, potential limitations of our study may be affected by population admixture and/or a Type I false positive errors due to the problem of multiple comparisons. We adjusted our analysis for ethnicity and the major associations remained significant. In terms of multiple comparisons, there are several possible methods of adjustment.

If we used a conservative Bonferroni correction and multiplied the P values by 32 for the number of analyzed SNPs, then only three associations remained significant (rs1477176 allelic association with leprosy susceptibility, and rs1477176 and rs8044354 dominant genotypic associations with leprosy susceptibility). However, because of linkage disequilibrium in genetic association studies, a strict Bonferroni correction is likely too conservative, and may increase Type II errors by falsely rejecting associations [33]. Furthermore, we found associations of multiple SNPs with leprosy (8/32), RR (7/32), and ENL (7/32). The occurrence of multiple SNP associations with these outcomes raises the likelihood that the associations remain statistically significant after adjusting for multiple comparisons. While these data are suggestive of an association between this gene region and leprosy reactive states, these findings will ultimately need to be verified in a separate population.

There are several possible mechanisms by which *NOD2* polymorphisms may influence development of reactive states. RR and ENL are immunologic/inflammatory states of leprosy that represent augmented Th1 (RR) or increased Th2/TNF-α (ENL) responses that occur during the course of disease or even years after treatment [35-37]. In animal studies and *ex vivo* experiments *NOD2* stimulation has been shown to augment both Th1 and Th2-dependent responses [38,39]. Therefore *NOD2* may augment Th1 or Th2 responses by direct recognition of *M. leprae* antigens. Interestingly, *M. leprae* contains a unique peptidoglycan structure, which may stimulate NOD2 differently and contribute to the unusual immunologic features of leprosy reactions [40]. In addition, others have also shown that *NOD2* inhibits TLR2 mediated Th1 responses [41,42], and we have reported that TLR2/TLR1 stimulation is associated with leprosy RR [16,43]. Therefore, one effect of *NOD2* may be through inhibition of TLR2 signaling.

The polymorphisms examined in this study were clustered in the chromosomal region 16q12 near *NOD2* and two other immunologically important genes, *SLIC1* and *CYLD* [44]. *SLIC1* may be important in the regulation of P-selection glycoprotein ligand of (*PSGL1)*, a protein involved with macrophage-mediated recruitment of myeloid and activated lymphocytes to tissues [45]. In addition negative regulation of the immune system by *CYLD* may be important in pneumonia models [46]. The polymorphisms described herein associated with ENL and RR tended to cluster in the region between *SLIC1* and *NOD2*, suggesting that these polymorphisms may influence the expression of either the *SLIC1* or *NOD2* genes. Further functional studies need to be done to determine exactly which gene (*SLIC1, NOD2*, or *CYLD*), is associated with leprosy reactional states.

Several genetic studies have identified the association of *NOD2* mutations with Crohn's disease and Blau's disease [24,25,47] both characterized by altered inflammatory states with granulomatous pathology. However, the association of *NOD2* mutations or polymorphisms with infection has not been well studied. A mutation associated with Crohn's disease (the *NOD2*-3020insC allele) has been linked to sepsis in infants of very low birth weight [48]. Also, nonsynonomous *NOD2* polymorphisms have been associated with active TB in a cohort of African Americans in Houston [28]. These nonsynonomous SNPs were not included in our study due to their absence in the HapMap database Asian populations. Moreover, two previous studies found no association of *NOD2* polymorphisms with TB [49,50]. Although our study suggests an association of *NOD2* variation with *M. leprae* susceptibility, the functional effect of these SNPs on *NOD2* signaling is currently not known.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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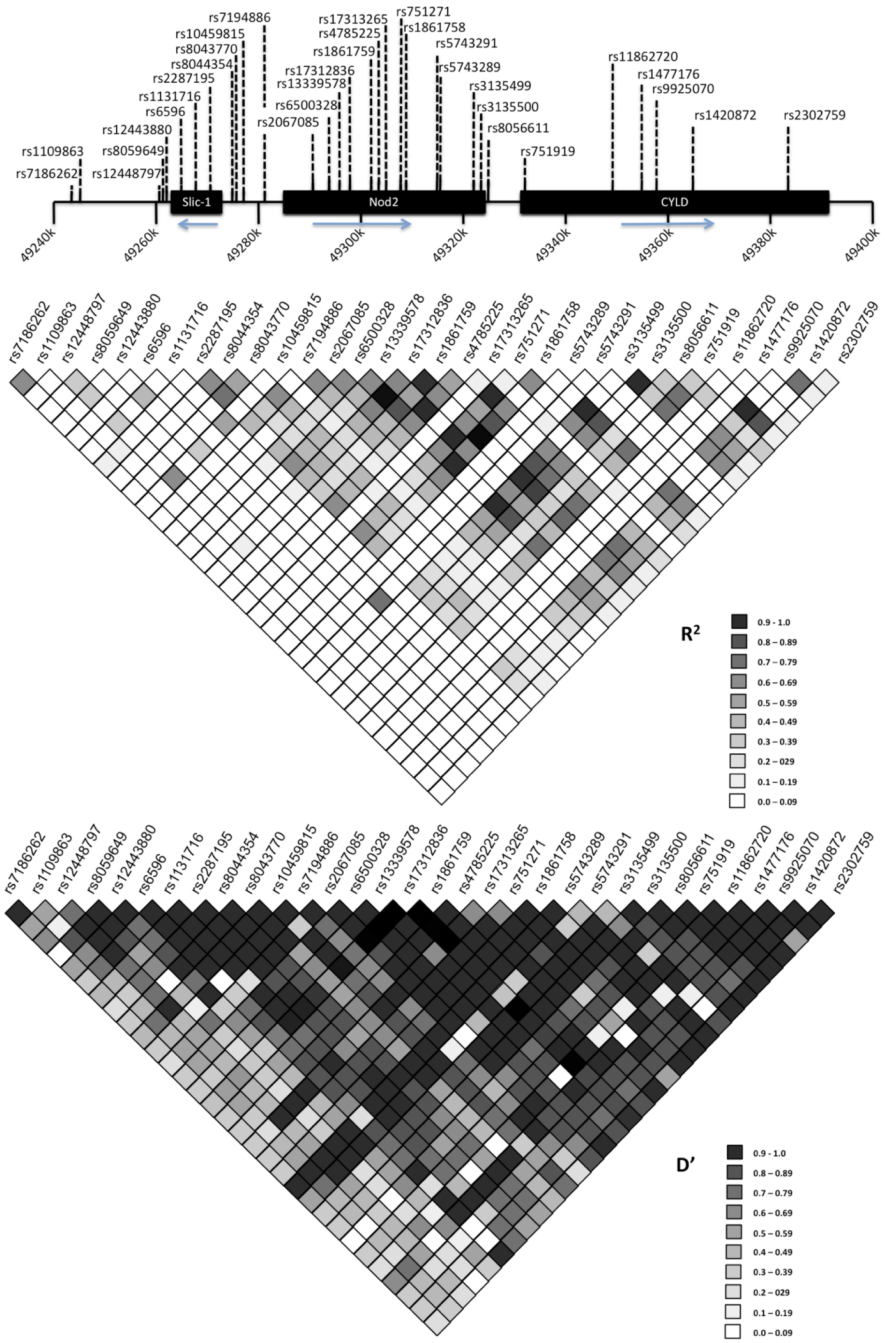

Figure 1.

Genetic map and Linkage disequilibrium (D' and R^2) of the single nucleotide polymorphisms used in the region of chromosome 16q21. Arrows represent direction of transcription

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Table 1

Population characteristics of Nepalese cohort

		ENL (among BL and LL patients only)		RR		Leprosy	
		Yes	**No**	**Yes**	**No**	**Yes**	**No**
	n=	124	428	240	693	933	101
Sex	M	89 (71.4)	305 (71.2)	164 (68.3)	488 (70.4)	652 (69.9)	63 (62.4)
	F	35 (28.2)	123 (28.7)	76 (31.7)	205 (29.6)	281 (30.1)	38 (37.6)
p[a]			0.911		0.544		0.121
Ethnicity (not including unassigned)	Brahmin	32 (31.4)	101 (27.6)	55 (26.6)	196 (32.2)	251 (30.3)	17 (19.1)
	Chetri	23 (22.6)	104 (28.4)	59 (28.5)	157 (25.8)	216 (26.4)	33 (31.5)
	Tamang	21 (20.6)	68 (18.6)	47 (22.7)	88 (14.5)	135 (17.0)	16 (18.0)
	Newar	6 (5.9)	33 (9.0)	15 (7.3)	55 (9.1)	70 (8.6)	20 (22.5)
	Magar	9 (8.8)	22 (6.0)	11 (5.3)	40 (6.6)	51 (6.4)	3 (3.4)
	Muslim	2 (2.0)	15 (4.1)	7 (3.4)	25 (4.1)	32 (3.9)	2 (2.3)
	Sarki	4 (3.9)	13 (3.6)	7 (3.4)	26 (4.3)	33 (4.2)	2 (2.3)
	Kami	5 (4.9)	10 (2.7)	6 (2.9)	21 (3.5)	27 (3.2)	1 (1.1)
p[b]		0.578		0.190		<0.01	
Age ± SD		37.7±13.6	46.0±16.4	38.09±15.6	46.3±16.1	44.2±16.4	31.9±12.9
p[c]			<0.01		<0.01		<0.01

[a] Statistical probability that frequency of sex is different between cases and controls (ENL, RR, leprosy).

[b] Statistical probability that ethnic frequency is different between cases (ENL, RR, leprosy) versus controls by 2×8 chi square analysis.

[c] Statistical probability that age is different in cases (ENL, RR, leprosy) versus controls by Wilcoxon rank-sum test.

Table 2

Allelic Association of *NOD2* Single Nucleotide Polymorphisms with Leprosy Disease Acquisition

		Allele		Genotype			Allelic Comparisons	
SNP,group	**Gene SNP type**	**0**	**1**	**00**	**01**	**11**	**OR (95%CI)**	**p*A***
rs7186262								
No Leprosy		100 (0.510)	96 (0.490)	25 (0.255)	50 (0.510)	23 (0.235)		
Leprosy		876 (0.519)	812 (0.481)	219 (0.259)	438 (0.519)	187 (0.222)	0.97 (0.71-1.31)	0.816
rs1109863								
No Leprosy		105 (0.520)	97 (0.480)	27 (0.276)	51 (0.520)	23 (0.235)		
Leprosy		816 (0.474)	906 (0.526)	186 (0.220)	444 (0.526)	231 (0.274)	1.20 (0.89-1.63)	0.216
rs12448797								
No Leprosy		188 (0.959)	8 (0.041)	91 (0.929)	6 (0.061)	1 (0.010)		
Leprosy		1583 (0.915)	147 (0.085)	727 (0.861)	129 (0.153)	9 (0.011)	**2.18 (1.06-5.23)**	**0.031**
rs8059649								
No Leprosy		194 (0.980)	4 (0.020)	95 (0.969)	4 (0.041)	0 (0.000)		
Leprosy		1682 (0.959)	72 (0.041)	807 (0.956)	68 (0.081)	2 (0.002)	2.08 (0.76-7.91)	0.151
rs12443880								
No Leprosy		194 (0.990)	2 (0.010)	96 (0.980)	2 (0.020)	0 (0.000)		
Leprosy		1677 (0.967)	57 (0.033)	813 (0.963)	51 (0.060)	3 (0.004)	3.30 (0.86-28.08)	0.081
rs6596								
No Leprosy	SLIC1	197 (0.995)	1 (0.005)	98 (1.000)	1 (0.010)	0 (0.000)		
Leprosy	Syn	1748 (0.984)	28 (0.016)	861 (1.020)	26 (0.031)	1 (0.001)	3.16 (0.52-129.66)	0.235
rs1131716								
No Leprosy	SLIC1	193 (0.985)	3 (0.015)	95 (0.969)	3 (0.031)	0 (0.000)		
Leprosy	NSyn	1681 (0.957)	75 (0.043)	805 (0.954)	71 (0.084)	2 (0.002)	2.87 (0.93-14.36)	0.063
rs2287195								
No Leprosy	SLIC1	144 (0.727)	54 (0.273)	55 (0.561)	34 (0.347)	10 (0.102)		
Leprosy	Intron	1053 (0.638)	597 (0.362)	333 (0.395)	387 (0.459)	105 (0.124)	**1.51 (1.08-2.14)**	**0.013**
rs8044354								
No Leprosy		128 (0.646)	70 (0.354)	46 (0.469)	36 (0.367)	17 (0.173)		
Leprosy		956 (0.545)	798 (0.455)	259 (0.307)	438 (0.519)	180 (0.213)	**1.53 (1.11-2.10)**	**0.006**

		Allele		Genotype			Allelic Comparisons	
SNP,group	**Gene**	**0**	**1**	**00**	**01**	**11**	**OR (95%CI)**	**p*A***
	SNP type							
rs8043770								
No Leprosy		148 (0.763)	46 (0.237)	61 (0.622)	26 (0.265)	10 (0.102)		
Leprosy		1235 (0.706)	515 (0.294)	439 (0.520)	357 (0.423)	79 (0.094)	1.34 (0.94-1.94)	0.095
rs10459815								
No Leprosy		192 (0.970)	6 (0.030)	93 (0.949)	6 (0.061)	0 (0.000)		
Leprosy		1632 (0.936)	112 (0.064)	766 (0.908)	100 (0.118)	6 (0.007)	2.20 (0.96-6.19)	0.058
rs7194886								
No Leprosy		160 (0.808)	38 (0.192)	68 (0.694)	24 (0.245)	7 (0.071)		
Leprosy		1415 (0.800)	353 (0.200)	568 (0.673)	279 (0.331)	37 (0.044)	1.05 (0.72-1.57)	0.796
rs2067085								
No Leprosy	NOD2	165 (0.825)	35 (0.175)	72 (0.735)	21 (0.214)	7 (0.071)		
Leprosy	Syn	1463 (0.838)	283 (0.162)	616 (0.730)	231 (0.274)	26 (0.031)	0.91 (0.61-1.38)	0.640
rs6500328								
No Leprosy	NOD2	151 (0.770)	45 (0.230)	63 (0.643)	25 (0.255)	10 (0.102)		
Leprosy	Intron	1325 (0.769)	397 (0.231)	503 (0.596)	319 (0.378)	39 (0.046)	1.01 (0.70-1.46)	0.976
rs13339578								
No Leprosy	NOD2	145 (0.732)	53 (0.268)	58 (0.592)	29 (0.296)	12 (0.122)		
Leprosy	Intron	1154 (0.683)	536 (0.317)	382 (0.453)	390 (0.462)	73 (0.086)	1.27 (0.90-1.80)	0.155
rs17312836								
No Leprosy	NOD2	153 (0.773)	45 (0.227)	64 (0.653)	25 (0.255)	10 (0.102)		
Leprosy	Intron	1380 (0.789)	370 (0.211)	539 (0.639)	302 (0.358)	34 (0.040)	0.91 (0.64-1.33)	0.606
rs1861759								
No Leprosy	NOD2	154 (0.778)	44 (0.222)	65 (0.663)	24 (0.245)	10 (0.102)		
Leprosy	Syn	1403 (0.789)	375 (0.211)	547 (0.648)	309 (0.366)	33 (0.039)	0.94 (0.65-1.37)	0.712
rs4785225								
No Leprosy	NOD2	145 (0.732)	53 (0.268)	58 (0.592)	29 (0.296)	12 (0.122)		
Leprosy	Intron	1227 (0.685)	563 (0.315)	411 (0.487)	405 (0.480)	79 (0.094)	1.26 (0.89-1.78)	0.176
rs17313265								
No Leprosy	NOD2	183 (0.915)	17 (0.085)	84 (0.857)	15 (0.153)	1 (0.010)		
Leprosy	Intron	1532 (0.894)	182 (0.106)	691 (0.819)	150 (0.178)	16 (0.019)	1.28 (0.75-2.30)	0.353

		Allele		Genotype			Allelic Comparisons	
SNP,group	**Gene**	**0**	**1**	**00**	**01**	**11**	**OR (95%CI)**	**p*A***
	SNP type							
rs751271								
No Leprosy	NOD2	147 (0.735)	53 (0.265)	59 (0.602)	29 (0.296)	12 (0.122)		
Leprosy	Intron	1227 (0.688)	557 (0.312)	415 (0.492)	397 (0.470)	80 (0.095)	1.26 (0.90-1.79)	0.170
rs1861758								
No Leprosy	NOD2	152 (0.784)	42 (0.216)	65 (0.663)	22 (0.224)	10 (0.102)		
Leprosy	Intron	1323 (0.777)	379 (0.223)	506 (0.600)	311 (0.368)	34 (0.040)	1.04 (0.72-1.52)	0.844
rs5743289								
No Leprosy	NOD2	195 (0.995)	1 (0.005)	97 (0.990)	1 (0.010)	0 (0.000)		
Leprosy	Intron	1731 (0.982)	31 (0.018)	851 (1.008)	29 (0.034)	1 (0.001)	3.49 (0.58-143.02)	0.191
rs5743291								
No Leprosy	NOD2	192 (0.980)	4 (0.020)	94 (0.959)	4 (0.041)	0 (0.000)		
Leprosy	NSyn	1679 (0.982)	31 (0.018)	825 (0.977)	29 (0.034)	1 (0.001)	0.89 (0.31-3.49)	0.822
rs3135499								
No Leprosy	NOD2	155 (0.783)	43 (0.217)	66 (0.673)	23 (0.235)	10 (0.102)		
Leprosy	3′UTR	1351 (0.762)	421 (0.238)	505 (0.598)	341 (0.404)	40 (0.047)	1.12 (0.78-1.64)	0.521
rs3135500								
No Leprosy	NOD2	155 (0.783)	43 (0.217)	66 (0.673)	23 (0.235)	10 (0.102)		
Leprosy	3′UTR	1311 (0.773)	385 (0.227)	502 (0.595)	307 (0.364)	39 (0.046)	1.06 (0.74-1.55)	0.754
rs8056611								
No Leprosy		119 (0.601)	79 (0.399)	40 (0.408)	39 (0.398)	20 (0.204)		
Leprosy		993 (0.573)	741 (0.427)	293 (0.347)	407 (0.482)	167 (0.198)	1.12 (0.82-1.54)	0.445
rs751919								
No Leprosy		148 (0.755)	48 (0.245)	60 (0.612)	28 (0.286)	10 (0.102)		
Leprosy		1291 (0.759)	411 (0.241)	487 (0.577)	317 (0.376)	47 (0.056)	0.98 (0.69-1.42)	0.916
rs11862720								
No Leprosy	CYLD	164 (0.854)	28 (0.146)	71 (0.724)	22 (0.224)	3 (0.031)		
Leprosy	Intron	1347 (0.809)	319 (0.191)	544 (0.645)	259 (0.307)	30 (0.036)	1.39 (0.91-2.19)	0.124
rs1477176								
No Leprosy	CYLD	169 (0.854)	29 (0.146)	73 (0.745)	23 (0.235)	3 (0.031)		
Leprosy	Intron	1571 (0.930)	119 (0.070)	733 (0.868)	105 (0.124)	7 (0.008)	**0.44 (0.28-0.71)**	**0.000**

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		Allele		Genotype			Allelic Comparisons	
SNP,group	**Gene SNP type**	**0**	**1**	**00**	**01**	**11**	**OR (95%CI)**	**p*A***
rs9925070								
No Leprosy	CYLD	151 (0.763)	47 (0.237)	62 (0.633)	27 (0.276)	10 (0.102)		
Leprosy	Intron	1363 (0.779)	387 (0.221)	527 (0.624)	309 (0.366)	39 (0.046)	0.91 (0.64-1.32)	0.603
rs1420872								
No Leprosy	CYLD	151 (0.786)	41 (0.214)	63 (0.643)	25 (0.255)	8 (0.082)		
Leprosy	Intron	1311 (0.785)	359 (0.215)	508 (0.602)	295 (0.350)	32 (0.038)	1.01 (0.69-1.49)	0.964
rs2302759								
No Leprosy	CYLD	130 (0.663)	66 (0.337)	46 (0.469)	38 (0.388)	14 (0.143)		
Leprosy	Intron	1141 (0.654)	603 (0.346)	382 (0.453)	377 (0.447)	113 (0.134)	1.04 (0.75-1.45)	0.801

*A*P values in bold are less than 0.05 for the allelic analyses

Table 3

Allelic and Genotypic Association of *NOD2* Single Nucleotide Polymorphisms with Type 1 Reversal Reaction in Leprosy

		Allele		Genotype			Allelic Comparisons		Genotypic Comparisons	
SNP,group	**Gene SNP type**	**0**	**1**	**00**	**01**	**11**	**OR (95%CI)**	**p*A***	**c^2 (2df)**	**P**
rs7186262										
No RR		640 (0.508)	620 (0.492)	154 (0.244)	332 (0.527)	144 (0.229)				
RR		236 (0.551)	192 (0.449)	65 (0.304)	106 (0.495)	43 (0.201)	1.19 (0.95-1.49)	0.120	3.024	0.221
rs1109863										
No RR		664 (0.516)	622 (0.484)	163 (0.259)	338 (0.537)	142 (0.225)				
RR		242 (0.555)	194 (0.445)	68 (0.318)	106 (0.495)	44 (0.206)	1.17 (0.93-1.46)	0.162	2.834	0.242
rs12448797										
No RR		1169 (0.912)	113 (0.088)	534 (0.848)	101 (0.160)	6 (0.010)				
RR		414 (0.924)	34 (0.076)	193 (0.902)	28 (0.131)	3 (0.014)	1.18 (0.78-1.81)	0.423	1.601	0.449
rs8059649										
No RR		1248 (0.960)	52 (0.040)	600 (0.952)	48 (0.076)	2 (0.003)				
RR		434 (0.956)	20 (0.044)	207 (0.967)	20 (0.093)	0 (0.000)	0.90 (0.52-1.62)	0.708	1.163	0.559
rs12443880										
No RR		1246 (0.967)	42 (0.033)	604 (0.959)	38 (0.060)	2 (0.003)				
RR		431 (0.966)	15 (0.034)	209 (0.977)	13 (0.061)	1 (0.005)	0.97 (0.52-1.90)	0.917	0.092	0.955
rs6596										
No RR	SLIC1	1292 (0.983)	22 (0.017)	636 (1.010)	20 (0.032)	1 (0.002)				
RR	Syn	456 (0.987)	6 (0.013)	225 (1.051)	6 (0.028)	0 (0.000)	1.29 (0.50-3.93)	0.577	0.474	0.789
rs1131716										
No RR	SLIC1	1251 (0.958)	55 (0.042)	600 (0.952)	51 (0.081)	2 (0.003)				
RR	NSyn	430 (0.956)	20 (0.044)	205 (0.958)	20 (0.093)	0 (0.000)	0.95 (0.55-1.68)	0.833	0.941	0.625
rs2287195										
No RR	SLIC1	760 (0.621)	464 (0.379)	233 (0.370)	294 (0.467)	85 (0.135)				
RR	Intron	293 (0.688)	133 (0.312)	100 (0.467)	93 (0.435)	20 (0.093)	**1.34 (1.06-1.72)**	**0.013**	**6.243**	**0.044**
rs8044354										
No RR		682 (0.525)	616 (0.475)	178 (0.283)	326 (0.517)	145 (0.230)				

SNP,group	Gene SNP type	Allele 0	Allele 1	Genotype 00	Genotype 01	Genotype 11	Allelic Comparisons OR (95%CI)	Allelic Comparisons p^A	Genotypic Comparisons c^2 (2df)	Genotypic Comparisons P
RR rs8043770		274 (0.601)	182 (0.399)	81 (0.379)	112 (0.523)	35 (0.164)	**1.36 (1.09-1.70)**	**0.005**	**7.807**	**0.020**
No RR		898 (0.690)	404 (0.310)	310 (0.492)	278 (0.441)	63 (0.100)				
RR rs10459815		337 (0.752)	111 (0.248)	129 (0.603)	79 (0.369)	16 (0.075)	**1.37 (1.06-1.76)**	**0.012**	**6.746**	**0.034**
No RR		1215 (0.938)	81 (0.063)	572 (0.908)	71 (0.113)	5 (0.008)				
RR rs7194886		417 (0.931)	31 (0.069)	194 (0.907)	29 (0.136)	1 (0.005)	0.90 (0.58-1.43)	0.618	0.883	0.643
No RR		1031 (0.788)	277 (0.212)	406 (0.644)	219 (0.348)	29 (0.046)				
RR rs2067085		384 (0.835)	76 (0.165)	162 (0.757)	60 (0.280)	8 (0.037)	**1.36 (1.02-1.82)**	**0.032**	5.172	0.075
No RR	NOD2	1073 (0.829)	221 (0.171)	445 (0.706)	183 (0.290)	19 (0.030)				
RR rs6500328	Syn	390 (0.863)	62 (0.137)	171 (0.799)	48 (0.224)	7 (0.033)	1.30 (0.95-1.79)	0.095	4.282	0.118
No RR	NOD2	973 (0.761)	305 (0.239)	362 (0.575)	249 (0.395)	28 (0.044)				
RR rs13339578	Intron	352 (0.793)	92 (0.207)	141 (0.659)	70 (0.327)	11 (0.051)	1.20 (0.92-1.58)	0.175	3.906	0.142
No RR	NOD2	849 (0.673)	413 (0.327)	275 (0.437)	299 (0.475)	57 (0.090)				
RR rs17312836	Intron	305 (0.713)	123 (0.287)	107 (0.500)	91 (0.425)	16 (0.075)	1.21 (0.94-1.55)	0.126	2.723	0.256
No RR	NOD2	1009 (0.780)	285 (0.220)	388 (0.616)	233 (0.370)	26 (0.041)				
RR rs1861759	Intron	371 (0.814)	85 (0.186)	151 (0.706)	69 (0.322)	8 (0.037)	1.23 (0.94-1.63)	0.128	2.799	0.247
No RR	NOD2	1023 (0.777)	293 (0.223)	390 (0.619)	243 (0.386)	25 (0.040)				
RR rs4785225	Syn	380 (0.823)	82 (0.177)	157 (0.734)	66 (0.308)	8 (0.037)	**1.33 (1.01-1.76)**	**0.041**	5.590	0.061
No RR	NOD2	893 (0.673)	433 (0.327)	290 (0.460)	313 (0.497)	60 (0.095)				
RR rs17313265	Intron	334 (0.720)	130 (0.280)	121 (0.565)	92 (0.430)	19 (0.089)	1.25 (0.98-1.59)	0.064	4.961	0.084

SNP,group	Gene SNP type	Allele 0	Allele 1	Genotype 00	Genotype 01	Genotype 11	Allelic Comparisons OR (95%CI)	Allelic Comparisons p[A]	Genotypic Comparisons c^2 (2df)	Genotypic Comparisons P
No RR	NOD2	1141 (0.893)	137 (0.107)	515 (0.817)	111 (0.176)	13 (0.021)				
RR	Intron	391 (0.897)	45 (0.103)	176 (0.822)	39 (0.182)	3 (0.014)	1.04 (0.72-1.53)	0.815	0.403	0.818
rs751271										
No RR	NOD2	892 (0.677)	426 (0.323)	293 (0.465)	306 (0.486)	60 (0.095)				
RR	Intron	335 (0.719)	131 (0.281)	122 (0.570)	91 (0.425)	20 (0.093)	1.22 (0.96-1.55)	0.092	4.466	0.107
rs1861758										
No RR	NOD2	981 (0.769)	295 (0.231)	369 (0.586)	243 (0.386)	26 (0.041)				
RR	Intron	342 (0.803)	84 (0.197)	137 (0.640)	68 (0.318)	8 (0.037)	1.22 (0.93-1.63)	0.144	2.829	0.243
rs5743289										
No RR	NOD2	1286 (0.982)	24 (0.018)	632 (1.003)	22 (0.035)	1 (0.002)				
RR	Intron	445 (0.985)	7 (0.015)	219 (1.023)	7 (0.033)	0 (0.000)	1.19 (0.49-3.28)	0.693	0.383	0.826
rs5743291										
No RR	NOD2	1255 (0.980)	25 (0.020)	616 (0.978)	23 (0.037)	1 (0.002)				
RR	NSyn	424 (0.986)	6 (0.014)	209 (0.977)	6 (0.028)	0 (0.000)	1.41 (0.56-4.22)	0.453	0.657	0.720
rs3135499										
No RR	NOD2	988 (0.754)	322 (0.246)	362 (0.575)	264 (0.419)	29 (0.046)				
RR	3′UTR	363 (0.786)	99 (0.214)	143 (0.668)	77 (0.360)	11 (0.051)	1.20 (0.92-1.56)	0.171	3.519	0.172
rs3135500										
No RR	NOD2	972 (0.769)	292 (0.231)	368 (0.584)	236 (0.375)	28 (0.044)				
RR	3′UTR	339 (0.785)	93 (0.215)	134 (0.626)	71 (0.332)	11 (0.051)	1.10 (0.84-1.44)	0.500	1.437	0.487
rs8056611										
No RR		726 (0.562)	566 (0.438)	210 (0.333)	306 (0.486)	130 (0.206)				
RR		267 (0.604)	175 (0.396)	83 (0.388)	101 (0.472)	37 (0.173)	1.19 (0.95-1.49)	0.122	2.317	0.314
rs751919										
No RR		949 (0.752)	313 (0.248)	353 (0.560)	243 (0.386)	35 (0.056)				
RR		342 (0.777)	98 (0.223)	134 (0.626)	74 (0.346)	12 (0.056)	1.15 (0.88-1.51)	0.286	1.746	0.418
rs11862720										
No RR	CYLD	997 (0.805)	241 (0.195)	401 (0.637)	195 (0.310)	23 (0.037)				
RR	Intron	350 (0.818)	78 (0.182)	143 (0.668)	64 (0.299)	7 (0.033)	1.08 (0.81-1.46)	0.573	0.319	0.853

		Allele		Genotype			Allelic Comparisons		Genotypic Comparisons	
SNP,group	**Gene SNP type**	**0**	**1**	**00**	**01**	**11**	**OR (95%CI)**	**p*A***	**c^2 (2df)**	**P**
rs1477176										
No RR	CYLD	1173 (0.929)	89 (0.071)	548 (0.870)	77 (0.122)	6 (0.010)				
RR	Intron	398 (0.930)	30 (0.070)	185 (0.864)	28 (0.131)	1 (0.005)	1.01 (0.65-1.60)	0.976	0.554	0.758
rs9925070										
No RR	CYLD	997 (0.770)	297 (0.230)	381 (0.605)	235 (0.373)	31 (0.049)				
RR	Intron	366 (0.803)	90 (0.197)	146 (0.682)	74 (0.346)	8 (0.037)	1.21 (0.92-1.60)	0.155	2.077	0.354
rs1420872										
No RR	CYLD	971 (0.781)	273 (0.219)	375 (0.595)	221 (0.351)	26 (0.041)				
RR	Intron	340 (0.798)	86 (0.202)	133 (0.621)	74 (0.346)	6 (0.028)	1.11 (0.84-1.48)	0.446	0.918	0.632
rs2302759										
No RR	CYLD	860 (0.663)	438 (0.337)	292 (0.463)	276 (0.438)	81 (0.129)				
RR	Intron	281 (0.630)	165 (0.370)	90 (0.421)	101 (0.472)	32 (0.150)	0.87 (0.69-1.09)	0.213	1.554	0.460

*A*SNPs are arranged by order that they are located on the chromosome.

*B*P values in bold are less than 0.05 for the allelic and genotypic analyses.

Table 4

Allelic and Genotypic Association of *NOD2* Single Nucleotide Polymorphisms with Erythema Nodosum Leprosum

		Allele		Genotype			Allelic Comparisons		Genotypic Comparisons	
SNP,group	**Gene SNP type**	**0**	**1**	**00**	**01**	**11**	**OR (95%CI)**	**p*A***	**c^2 (2df)**	**P**
rs7186262										
No RR		394 (0.520)	364 (0.480)	99 (0.261)	196 (0.517)	84 (0.222)				
RR		115 (0.513)	109 (0.487)	30 (0.268)	55 (0.491)	27 (0.241)	0.97 (0.72-1.33)	0.866	0.274	0.872
rs1109863										
No RR		395 (0.506)	385 (0.494)	99 (0.261)	197 (0.520)	94 (0.248)				
RR		124 (0.539)	106 (0.461)	35 (0.313)	54 (0.482)	26 (0.232)	1.14 (0.84-1.55)	0.383	1.163	0.559
rs12448797										
No RR		719 (0.908)	73 (0.092)	331 (0.873)	57 (0.150)	8 (0.021)				
RR		211 (0.894)	25 (0.106)	93 (0.830)	25 (0.223)	0 (0.000)	0.86 (0.52-1.45)	0.528	5.264	0.072
rs8059649										
No RR		774 (0.965)	28 (0.035)	373 (0.984)	28 (0.074)	0 (0.000)				
RR		224 (0.941)	14 (0.059)	105 (0.938)	14 (0.125)	0 (0.000)	0.58 (0.29-1.21)	0.100	2.827	0.093
rs12443880										
No RR		763 (0.961)	31 (0.039)	368 (0.971)	27 (0.071)	2 (0.005)				
RR		222 (0.965)	8 (0.035)	107 (0.955)	8 (0.071)	0 (0.000)	1.13 (0.50-2.88)	0.766	0.584	0.747
rs6596										
No RR	SLIC1	805 (0.984)	13 (0.016)	396 (1.045)	13 (0.034)	0 (0.000)				
RR	Syn	237 (0.988)	3 (0.013)	117 (1.045)	3 (0.027)	0 (0.000)	1.28 (0.35-7.03)	0.705	0.146	0.703
rs1131716										
No RR	SLIC1	765 (0.949)	41 (0.051)	363 (0.958)	39 (0.103)	1 (0.003)				
RR	NSyn	223 (0.945)	13 (0.055)	105 (0.938)	13 (0.116)	0 (0.000)	0.92 (0.47-1.90)	0.797	0.469	0.791
rs2287195										
No RR	SLIC1	466 (0.635)	268 (0.365)	154 (0.406)	158 (0.417)	55 (0.145)				
RR	Intron	128 (0.587)	90 (0.413)	31 (0.277)	66 (0.589)	12 (0.107)	0.82 (0.59-1.13)	0.202	**10.366**	**0.006**
rs8044354										
No RR		443 (0.548)	365 (0.452)	125 (0.330)	193 (0.509)	86 (0.227)				

SNP,group	Gene SNP type	Allele 0	Allele 1	Genotype 00	Genotype 01	Genotype 11	Allelic Comparisons OR (95%CI)	Allelic Comparisons p^A	Genotypic Comparisons c^2 (2df)	Genotypic Comparisons P
RR		111 (0.474)	123 (0.526)	20 (0.179)	71 (0.634)	26 (0.232)	**0.74 (0.55-1.01)**	**0.046**	**9.272**	**0.010**
rs8043770										
No RR		569 (0.706)	237 (0.294)	205 (0.541)	159 (0.420)	39 (0.103)				
RR		158 (0.675)	76 (0.325)	50 (0.446)	58 (0.518)	9 (0.080)	0.87 (0.63-1.20)	0.367	3.835	0.147
rs10459815										
No RR		746 (0.933)	54 (0.068)	350 (0.923)	46 (0.121)	4 (0.011)				
RR		215 (0.919)	19 (0.081)	98 (0.875)	19 (0.170)	0 (0.000)	0.82 (0.47-1.50)	0.472	2.933	0.231
rs7194886										
No RR		663 (0.813)	153 (0.188)	275 (0.726)	113 (0.298)	20 (0.053)				
RR		180 (0.756)	58 (0.244)	66 (0.589)	48 (0.429)	5 (0.045)	0.72 (0.50-1.03)	0.057	**6.943**	**0.031**
rs2067085										
No RR	NOD2	674 (0.845)	124 (0.155)	289 (0.763)	96 (0.253)	14 (0.037)				
RR	Syn	186 (0.802)	46 (0.198)	74 (0.661)	38 (0.339)	4 (0.036)	0.74 (0.50-1.11)	0.121	3.566	0.168
rs6500328										
No RR	NOD2	607 (0.778)	173 (0.222)	239 (0.631)	129 (0.340)	22 (0.058)				
RR	Intron	170 (0.720)	66 (0.280)	57 (0.509)	56 (0.500)	5 (0.045)	0.73 (0.52-1.04)	0.066	**8.098**	**0.017**
rs13339578										
No RR	NOD2	520 (0.679)	246 (0.321)	173 (0.456)	174 (0.459)	36 (0.095)				
RR	Intron	146 (0.646)	80 (0.354)	41 (0.366)	64 (0.571)	8 (0.071)	0.86 (0.63-1.20)	0.356	4.410	0.110
rs17312836										
No RR	NOD2	640 (0.804)	156 (0.196)	260 (0.686)	120 (0.317)	18 (0.047)				
RR	Intron	172 (0.741)	60 (0.259)	61 (0.545)	50 (0.446)	5 (0.045)	**0.70 (0.49-1.00)**	**0.039**	**6.900**	**0.032**
rs1861759										
No RR	NOD2	650 (0.797)	166 (0.203)	261 (0.689)	128 (0.338)	19 (0.050)				
RR	Syn	176 (0.733)	64 (0.267)	60 (0.536)	56 (0.500)	4 (0.036)	**0.70 (0.50-1.00)**	**0.037**	**9.574**	**0.008**
rs4785225										
No RR	NOD2	564 (0.683)	262 (0.317)	190 (0.501)	184 (0.485)	39 (0.103)				
RR	Intron	157 (0.654)	83 (0.346)	45 (0.402)	67 (0.598)	8 (0.071)	0.88 (0.64-1.21)	0.404	4.851	0.088
rs17313265										

SNP,group	Gene SNP type	Allele 0	Allele 1	Genotype 00	Genotype 01	Genotype 11	Allelic Comparisons OR (95%CI)	Allelic Comparisons p[A]	Genotypic Comparisons c^2 (2df)	Genotypic Comparisons P
No RR	NOD2	676 (0.873)	98 (0.127)	299 (0.789)	78 (0.206)	10 (0.026)				
RR	Intron	211 (0.917)	19 (0.083)	96 (0.857)	19 (0.170)	0 (0.000)	1.61 (0.95-2.85)	0.068	4.013	0.134
rs751271										
No RR	NOD2	567 (0.688)	257 (0.312)	195 (0.515)	177 (0.467)	40 (0.106)				
RR	Intron	156 (0.655)	82 (0.345)	45 (0.402)	66 (0.589)	8 (0.071)	0.86 (0.63-1.19)	0.341	5.913	0.052
rs1861758										
No RR	NOD2	604 (0.791)	160 (0.209)	240 (0.633)	124 (0.327)	18 (0.047)				
RR	Intron	169 (0.728)	63 (0.272)	58 (0.518)	53 (0.473)	5 (0.045)	**0.71 (0.50-1.01)**	**0.047**	**6.859**	**0.032**
rs5743289										
No RR	NOD2	803 (0.984)	13 (0.016)	395 (1.042)	13 (0.034)	0 (0.000)				
RR	Intron	234 (0.983)	4 (0.017)	115 (1.027)	4 (0.036)	0 (0.000)	0.95 (0.29-4.03)	0.925	0.009	0.924
rs5743291										
No RR	NOD2	751 (0.978)	17 (0.022)	368 (0.971)	15 (0.040)	1 (0.003)				
RR	NSyn	230 (0.983)	4 (0.017)	113 (1.009)	4 (0.036)	0 (0.000)	1.30 (0.42-5.37)	0.637	0.366	0.833
rs3135499										
No RR	NOD2	627 (0.768)	189 (0.232)	240 (0.633)	147 (0.388)	21 (0.055)				
RR	3′UTR	171 (0.718)	67 (0.282)	58 (0.518)	55 (0.491)	6 (0.054)	0.77 (0.55-1.08)	0.114	4.154	0.125
rs3135500										
No RR	NOD2	588 (0.776)	170 (0.224)	230 (0.607)	128 (0.338)	21 (0.055)				
RR	3′UTR	166 (0.735)	60 (0.265)	59 (0.527)	48 (0.429)	6 (0.054)	0.80 (0.56-1.15)	0.199	2.916	0.233
rs8056611										
No RR		450 (0.568)	342 (0.432)	137 (0.361)	176 (0.464)	83 (0.219)				
RR		126 (0.525)	114 (0.475)	32 (0.286)	62 (0.554)	26 (0.232)	0.84 (0.62-1.13)	0.238	2.831	0.243
rs751919										
No RR		596 (0.766)	182 (0.234)	231 (0.609)	134 (0.354)	24 (0.063)				
RR		165 (0.730)	61 (0.270)	58 (0.518)	49 (0.438)	6 (0.054)	0.83 (0.58-1.18)	0.266	3.005	0.223
rs11862720										
No RR	CYLD	612 (0.799)	154 (0.201)	242 (0.639)	128 (0.338)	13 (0.034)				
RR	Intron	186 (0.809)	44 (0.191)	72 (0.643)	42 (0.375)	1 (0.009)	1.06 (0.72-1.58)	0.745	2.259	0.323

SNP,group	Gene SNP type	Allele 0	Allele 1	Genotype 00	Genotype 01	Genotype 11	Allelic Comparisons OR (95%CI)	Allelic Comparisons p^A	Genotypic Comparisons c^2 (2df)	Genotypic Comparisons P
rs1477176										
No RR	CYLD	704 (0.936)	48 (0.064)	329 (0.868)	46 (0.121)	1 (0.003)				
RR	Intron	219 (0.952)	11 (0.048)	104 (0.929)	11 (0.098)	0 (0.000)	1.36 (0.68-2.95)	0.371	0.932	0.627
rs9925070										
No RR	CYLD	632 (0.790)	168 (0.210)	252 (0.665)	128 (0.338)	20 (0.053)				
RR	Intron	180 (0.756)	58 (0.244)	65 (0.580)	50 (0.446)	4 (0.036)	0.82 (0.58-1.18)	0.269	4.270	0.118
rs1420872										
No RR	CYLD	587 (0.789)	157 (0.211)	230 (0.607)	127 (0.335)	15 (0.040)				
RR	Intron	174 (0.770)	52 (0.230)	66 (0.589)	42 (0.375)	5 (0.045)	0.89 (0.62-1.31)	0.541	0.427	0.808
rs2302759										
No RR	CYLD	521 (0.651)	279 (0.349)	180 (0.475)	161 (0.425)	59 (0.156)				
RR	Intron	156 (0.661)	80 (0.339)	50 (0.446)	56 (0.500)	12 (0.107)	1.04 (0.76-1.44)	0.782	2.666	0.264

[A] P values in bold are less than 0.05 for the allelic and genotypic analyses