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Toll-Like Receptor 2 *(TLR2)* Polymorphisms Are Associated with Reversal Reaction in Leprosy

Pierre-Yves Bochud[1], **Thomas R. Hawn**[1,2], **M. Ruby Siddiqui**[4,a], **Paul Saunderson**[5,a], **Sven Britton**[6,a], **Isaac Abraham**[6], **Azeb Tadesse Argaw**[6,a], **Marta Janer**[1], **Lue Ping Zhao**[3], **Gilla Kaplan**[4], and **Alan Aderem**[1,2]

[1] Institute for Systems Biology, University of Washington, Seattle

[2] Department of Medicine, University of Washington, Seattle

[3] Quantitative Genetic Epidemiology, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle

[4] Public Health Research Institute, Laboratory of Mycobacterial Immunity and Pathogenesis, University of Medicine and Dentistry of New Jersey, Newark

[5] All-Africa Leprosy Rehabilitation and Training Centre

[6] Armauer Hansen Research Institute, Addis Ababa, Ethiopia

Abstract

Background—Leprosy is characterized by a spectrum of clinical manifestations that depend on the type of immune response against the pathogen. Patients may undergo immunological changes known as “reactional states” (reversal reaction and erythema nodosum leprosum) that result in major clinical deterioration. The goal of the present study was to assess the effect of Toll-like receptor 2 *(TLR2)* polymorphisms on susceptibility to and clinical presentation of leprosy.

Methods—Three polymorphisms in *TLR2* (597C→T, 1350T→C, and a microsatellite marker) were analyzed in 431 Ethiopian patients with leprosy and 187 control subjects. The polymorphism-associated risk of developing leprosy, lepromatous (vs. tuberculoid) leprosy, and leprosy reactions was assessed by multivariate logistic regression models.

Results—The microsatellite and the 597C→T polymorphisms both influenced susceptibility to reversal reaction. Although the 597T allele had a protective effect (odds ratio [OR], 0.34 [95% confidence interval {CI}, 0.17–0.68]; $P = .002$ under the dominant model), homozygosity for the 280-bp allelic length of the microsatellite strongly increased the risk of reversal reaction (OR, 5.83 [95% CI, 1.98–17.15]; $P = .001$ under the recessive model). These associations were consistent among 3 different ethnic groups.

Conclusions—These data suggest a significant role for TLR-2 in the occurrence of leprosy reversal reaction and provide new insights into the immunogenetics of the disease.

Reprints or correspondence: Dr. Pierre-Yves Bochud, Institute for Systems Biology, 1441 N. 34th St., Seattle, WA 98103-8904 (pyb@systemsbiology.org).
[a]Present affiliations: Centre for Infections, Health Protection Agency, London, United Kingdom (M.R.S.); American Leprosy Missions, Greenville, South Carolina (P.S.); Department of Medicine, Karolinska Institute, Stockholm, Sweden (S.B.); Mount Sinai School of Medicine, New York, New York (A.T.A.).

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Leprosy is characterized by a polarized spectrum of clinical manifestations that correlate with the level of cell-mediated immune response against *Mycobacterium leprae* [1,2]. At one end, patients with tuberculoid leprosy manifest a strong cellular immune response with localized lesions that have few or no bacilli in well-formed granulomas with the expression of Th1 cytokines [2–4]. At the opposite end, patients with lepromatous leprosy have a suboptimal cellular immune response, disseminated infection with extensive lesions characterized by the presence of numerous intracellular bacilli, and the expression of Th2 cytokines. Many patients with leprosy have an unstable response to the pathogen and may undergo immunological changes known as "reactional states," resulting in major clinical alterations. Reversal reaction represents a sudden shift of the immune system, often toward the tuberculoid pole, with an increased cell-mediated response against *M. leprae* antigens that can rapidly lead to severe tissue damage [5]. Reversal reactions are observed more frequently among patients with borderline lepromatous leprosy, especially after the initiation of multidrug therapy, with an incidence rate ranging from 18% to 44% [6]. Although genetic studies have implicated several genes in susceptibility to leprosy, the genetic factors that predispose individuals to reversal reaction remain unknown [2,7–9].

Toll-like receptors (TLRs) are type 1 transmembrane proteins expressed in innate immune cells that play a critical role in the inflammatory response to microbial pathogens [10,11]. The extracellular domain comprises leucine-rich repeat structures that can recognize a vast range of microbial products. On activation, the intracellular domain elicits a complex signal transduction that ultimately activates the transcription of proinflammatory cytokines. These cytokines play an essential role in the host innate immune response and determine the activation of adaptive immune mechanisms [12]. We previously demonstrated that TLR-2 mediates the innate immune recognition of *M. leprae* [13]. Polymorphisms in *TLR2* have recently been found to be associated with susceptibility to several infectious diseases, including mycobacterial infections [14–18]. In this study, we investigate whether *TLR2* polymorphisms are associated with susceptibility to leprosy and/or leprosy reactions in a case-control study from Ethiopia. We demonstrate an association between *TLR2* polymorphisms and reversal reaction among patients with leprosy.

PATIENTS, MATERIALS, AND METHODS

Case-control study

Patients with a history of leprosy were drawn from AMFES (All-Africa Leprosy Rehabilitation and Training Multidrug Therapy Field Evaluation Study), a long-term study of fixed-duration multidrug therapy in Ethiopian patients with leprosy [19]. Ethnicity was self-reported and comprised 3 major ethnic groups (Oromo, Amhara, and Gurage). Healthy control subjects from each ethnic group were recruited from the local population (n = 197). The enrollment procedures and the administration of treatment have been described elsewhere [19,20]. Briefly, patients were enrolled at one of the clinics in the study area between 1988 and 1993 and were followed for 5 to 11 years after release from treatment (n = 441). Skin smears were done routinely, and the bacillary index and morphological index were recorded for each of 4 sites. Leprosy types were established on clinical grounds according to the simplified Ridley/Jopling classification, which adds the rarely occurring midborderline patients to the borderline lepromatous (BL) group [21]. A small number of patients were classified as multibacillary (MB) or paucibacillary (PB), according the World Health Organization classification (n = 25). Leprosy reactions were reported only in a subgroup of patients (n = 216). Reactions were diagnosed on the basis of clinical findings of signs of inflammation in leprosy skin lesions, which has been extensively described elsewhere [22,23]. Although reversal reaction can occasionally occur for the first time >5 years after the start of treatment, the vast majority occur within the first year, as has been shown in previous studies [22,23]. The patients who were assessed for leprosy reactions had

been followed for at least 8 years, to insure that all nonreactional patients with leprosy were truly nonreactional. Subjects were informed of the risks and benefits of the study and signed a consent form. Genomic DNA was extracted from whole blood by use of the Nucleon DNA Extraction Kit (Amersham Biosciences). Approval for human study protocols was obtained from the human subject review boards at the Armauer Hansen Research Institute, Rockefeller University, the Public Health Research Institute, the University of Washington, and the Western Institutional Review Board.

TLR2 *microsatellite*

The *TLR2* microsatellite marker is located between 162 and 100 bp upstream of the start codon of *TLR2* and contains 2 subsequent variable nucleotide tandem repeats (VNTRs), CT and TG. We amplified a fragment of the genomic DNA from the patients with leprosy and the control subjects comprising these 2 VNTRs by use of the primers 5′-TCCGGATGGTTGTGCTTTTAAGTACTGC-3′ and 5′-GTGGCATTGTCCAGTGCTTCAACC-3′. Samples were cycled using a Peltier Thermal Cycler (MJ Research). Polymerase chain reactions (PCRs) incorporated an infrared dye (IRD700-dATP; Enzo/LI-COR) and were analyzed on LI-COR 4200 automated sequencers (LI-COR Biosciences), as described elsewhere [24]. In-house genotyping software (SAGA; LI-COR Biosciences) was used to determine alleles. Calls were confirmed by manual checking. Analysis of a PCR product revealed 18 different allelic forms, with lengths ranging from 262 to 302 bp, corresponding to microsatellite lengths of 40 to 80 bp, respectively. The most frequent allelic lengths were 280 (33%), 282 (13%), 284 (13%), 288 (6%), and 290 (10%) bp, representing ~80% of the alleles. The microsatellite maker was in Hardy-Weinberg equilibrium among case patients and control subjects of the 3 ethnicities, excepted in Oromo case patients ($P = .03$). Allelic lengths with frequencies <5% were grouped together.

Single-nucleotide polymorphisms (SNPs)

Selection of SNPs for high throughput was performed using a public database of TLR SNPs (Innate Immunity Program in Genomic Applications database; available at: http://innateimmunity.net/) as well as SNPs reported in the literature [14,25] at the time genotyping was started. SNPs in the coding region of *TLR2* that induced amino acid change as well as synonymous SNPs with a frequency of >5% were selected for high-throughput genotyping in the whole population by use of Sequenom technology, as described elsewhere [26]. On the basis of these criteria, 4 SNPs were selected (597C→T, N199N, rs3804099; 1350T→C, S450S, rs3804100; 2029C→T, R677W; and 2258G→A, R753Q, rs5743708). However, only 2 synonymous SNPs were detected in the Ethiopian population (597C→T and 1350T→C); 597C→T and 1350T→C were in Hardy-Weinberg equilibrium among case patients and control subjects of the 3 ethnicities. The 2029C→T SNP that had been associated with the occurrence of lepromatous leprosy in a Korean population was subsequently found not to be a true *TLR2* polymorphism [25,27]. In fact, the C→T substitution is located in a pseudogene region highly homologous to the *TLR2* exon 3, which is located ~23 kb upstream from the *TLR2* gene [27]. Subsequent studies suggested that the 2258G→A [R753Q] SNP that was associated with tuberculosis in Turkey is restricted to white populations [14,28].

Haplotypes

Haplotypes including the *TLR2* microsatellite polymorphisms and the 597C→T and 1350T→C SNPs were inferred separately for each ethnic group by use of the expectation-maximization algorithm implemented in the DECIPHER program (S.A.G.E.) [29]. The most frequent haplotypes among all ethnic groups were 280-C-T (35%), 290-T-T (11%), 282-T-T

(9%), 284-C-T (8%), 288-T-T (6%), and 284-T-T (5%), representing ~75% of all haplotypes. Haplotypes with frequencies <5% were grouped together.

Statistical analysis

All analysis were performed using Stata (version 9; StataCorp). The risk associated with *TLR2* SNPs were assessed in general multivariate logistic regression models that did not assume any particular mode of inheritance and accounted for the presence of each genotype (0/1 and 1/1) versus the wild-type genotype (0/0; analysis by genotype). A similar method was used for multiallelic markers (microsatellite allelic lengths and haplotypes), but the reference (0/0) was the absence of marker (i.e., all patients who did not have a copy of the microsatellite allelic length or the haplotype). To assess associations of interest, we performed likelihood ratio tests for 3 different models (dominant, recessive, and additive) versus the general model. The best-fitting model was assumed in the final presentation of the results. All analyses were adjusted for age groups, sex, and ethnicity (when applicable). Because of the limited sample size, statistical analyses were initially performed in the whole population. Consistency of significant results was then verified within each ethnic group.

RESULTS

The general characteristics of the 441 case patients and 197 control subjects are shown in table 1. Both groups had similar male to female ratios. Mean age was significantly lower in control subjects than in case patients (mean ± SD, 29.1 ± 12.4 vs. 39.0 ± 15.1 years; $P < .001$). A total of 298 case patients (68%) were grouped into the lepromatous pole, including 199 case patients classified as BL, 81 classified as lepromatous lepromatous (LL), and 18 classified as MB. Of the 138 case patients grouped into the tuberculoid pole, most were classified as borderline tuberculoid (BT; $n = 128$). The remaining patients were classified as tuberculoid tuberculoid (TT; $n = 3$) or PB ($n = 7$).

Of the 441 patients, 216 had complete data on leprosy complications. Neuritis was the most common complication and occurred in 72% of BL patients, 69% of LL patients, and 49% of BT patients. Reversal reaction occurred almost exclusively among borderline patients (38% of BL patients, 28% of BT patients, and 3% of LL patients), and erythema nodosum leprosum was, as expected, found exclusively among lepromatous patients (19% of LL patients and 10% of BL patients). The frequency of *TLR2* polymorphisms was similar among the 3 ethnic groups (table 2). Pairwise linkage disequilibrium between *TLR2* SNPs and microsatellite allelic lengths are shown in table 3.

To investigate whether susceptibility to leprosy was associated with *TLR2* polymorphisms, we compared frequencies of *TLR2* polymorphisms among leprosy case patients in a general model, adjusting for sex, age groups, and ethnicity (table 4). There were no relevant differences in the frequencies of 597C→T and 1350T→C, but the 290-bp allelic length of the microsatellite marker was less frequent among leprosy case patients than control subjects (0 [0/0], 1 [0/1], and 2 [1/1] copies of the 290-bp allelic length were found in 82.8%, 16.2%, and 1.0% of leprosy case patients and in 74.9%, 23.5%, and 1.6% of control subjects; odds ratio [OR], 0.62 [95% confidence interval {CI}, 0.41–0.93]; $P = .02$, assuming an additive model, table 5). This association was stronger in the Amhara subjects (OR, 0.40 [95% CI, 0.17–0.93]; $P = .03$) than in the Oromo (OR, 0.66 [95% CI, 0.36–1.21]; $P = .18$) or the Gurage (OR, 0.61 [95% CI, 0.25–1.48]; $P = .27$) subjects (table 5).

We also compared frequencies of *TLR2* polymorphisms between patients with lepromatous leprosy and those with tuberculoid leprosy, adjusting for sex, age group, and ethnicity (table 4). The 288-bp allelic length was less frequent among patients with lepromatous leprosy than among those with tuberculoid leprosy (0 [0/0], 1 [0/1], and 2 [1/1] copies of the 288-bp

allelic length were found in 89.1%, 10.6%, and 0.4% of lepromatous case patients and in 80.0%, 20.0%, and 0% of tuberculoid case patients, respectively; OR, 0.49 [95% CI, 0.27 – 0.90]; *P* = .02, assuming a dominant model) (table 6). On the contrary, the 282-bp allele tended to be more frequent in the lepromatous group (0 [0/0], 1 [0/1], and 2 [1/1] copies of the 282-bp allelic length were found in 72.9%, 26.1%, and 1.1% of lepromatous case patients and in 81.5%, 16.2%, and 2.3% of tuberculoid case patients, respectively; OR, 1.64 [95% CI, 0.96–2.81]; *P* = .07, assuming a dominant model, table 6). These observations were consistent among each ethnic group, although statistical significance was not reached because of smaller sample sizes (table 6).

We then investigated whether *TLR2* polymorphisms were associated with leprosy reactions. Although no associations were found with neuritis or erythema nodosum leprosum (data not shown), reversal reaction was associated with the *TLR2* 597C→T SNP and the microsatellite marker (table 4). The presence of the 597T allele had a protective effect on reversal reaction (597 CC [0/0], CT [0/1], and TT [1/1] were found in 48.3%, 38.3%, and 13.3% of patients with reversal reaction and in 23.7%, 56.1%, and 16.5% of those without reversal reaction, respectively; OR, 0.34 [95% CI, 0.17–0.68]; *P* = .002, assuming a dominant model) (table 7). This association was consistent among the 3 ethnic groups, although the *P* value was not significant for the Gurage ethnicity because of reduced sample size (table 7). Homozygosity for the 280-bp allelic length of the microsatellite marker strongly increased the risk of reversal reaction (0 [0/0], 1 [0/1], and 2 [1/1] copies of the 280-bp allelic length were found in 30.5%, 47.5%, and 22.0% of patients with reversal reaction and in 49.6%, 44.7%, and 5.7% of those without reversal reaction, respectively; OR, 5.83 [95% CI, 1.98–17.15]; *P* = .001, assuming a recessive model (table 7). This observation was consistent among the 3 ethnic groups, even though the sample size among Gurage subjects was limited.

To determine whether coinheritance of the 3 *TLR2* microsatellite polymorphisms, 597C→T and 1350T→C, and the microsatellite further increased the risk of reversal reaction, we examined the association between *TLR2* haplotypes and reversal reaction (table 8). Homozygosity for a *TLR2* haplotype containing the 280-bp microsatellite allelic length, the 597C allele, and the 1350T allele (280-C-T) was more frequent among patients with reversal reaction than among other patients (0 [0/0], 1 [0/1], and 2 [1/1] copies of haplotype 280-C-T were found in 32.3%, 46.8%, and 21.0% of patients with reversal reaction and in 49.3%, 44.6%, and 6.1% of those without reversal reaction, respectively; OR, 6.39 [95% CI, 2.14–19.07]; *P* = .001, assuming a recessive model) (table 7). The 280-C-T haplotype had a slightly stronger effect on reversal reaction than the 280-bp allelic length alone (OR for the 280-bp allelic length alone, 5.83 [95% CI, 1.98–17.15]; *P* < .001) (OR for the 280-C-T haplotype, 6.39 [95% CI, 2.14–19.07]; *P* < .001). Again, the association was consistent among the 3 ethnic groups, even though the sample size for Gurage patients was small (only 2 patients had the 280-C-T/280-C-T diplotype, and both had reversal reaction) (table 7).

DISCUSSION

In the present article, we show a significant association between a *TLR2* microsatellite polymorphism as well as a *TLR2* SNP (597C→T) and the occurrence of reversal reaction among patients with leprosy. The observation is strengthened by the fact that the biological role played by TLR-2 in leprosy has been previously established and that the observations are consistent among 3 different ethnic groups.

Leprosy is characterized by a polarized immunological response ranging between the tuberculoid and the lepromatous poles, which vary in the level of cell-mediated immunity against *M. leprae*. Reactional states such as reversal reaction reflect dynamic changes in the immune response that provide insight into the immunopathogenesis of disease. Reversal

reaction represents a shift of the cellular immune response, often from the lepromatous to the tuberculoid form, usually occurring after starting treatment. Reversal reaction lesions contain infiltrating T cells producing a Th1 pattern of cytokines that is characteristic of cell-mediated immunity and is associated with increased killing of *M. leprae* [5]. The factors that trigger changes in the cytokine patterns in leprosy lesions remain largely unknown. On ligand binding, Toll-like receptors mediate maturation of dendritic cells, leading to the secretion of cytokines that can direct the adaptive T cell response toward either a Th1 or a Th2 pattern [12]. Recently, TLR-2 has been shown to be essential for the recognition of *M. leprae* and to be expressed in tuberculoid lesions [13,30]. These data suggest that TLR-2 may play a significant role in the changes in immune response that occur during reversal reaction.

Although little information is available on the role played by host genetic polymorphisms in susceptibility to reversal reaction, previous studies have shown associations between susceptibility to leprosy or leprosy types and polymorphisms in different genes [2,31–37]. We also found an association between certain allelic lengths of the *TLR2* microsatellite and susceptibility to leprosy or leprosy type, but these were not as strong as the association with reversal reaction. This suggests that *TLR2* is differentially involved in the clinical manifestation of leprosy and/or that mutations in other genes (such as *TLR1* or *TLR6*) may have a stronger effect on susceptibility to leprosy or leprosy type.

A Turkish study revealed an association between susceptibility to tuberculosis and the *TLR2* 2258G→A SNP (R753Q), which impairs TLR-2 signaling [14]. The 2258G→A SNP was not found among Ethiopian patients with leprosy and control subjects and seems to be restricted to white populations. In contrast with the 2258G→A SNP, the *TLR2* 597C→T SNP described here does not induce an amino acid change. This SNP may be in linkage disequilibrium with another functional SNP in *TLR2* or in a gene located nearby. Currently available genetic databases do not contain a *TLR2* nonsynonymous SNP in linkage disequilibrium with 597C→T, but one cannot exclude the possibility that such a polymorphism exists in certain populations. This SNP may also be in linkage disequilibrium with another SNP located in the *TLR2* promoter or at the 3′ end of mRNA and thereby influence promoter activity or the stability of the transcript. In the same way, different allelic lengths of the *TLR2* microsatellite marker were shown to have functional consequences. A recent study indicated that length variation in the GT repeat of the *TLR2* microsatellite marker influences promoter activity and suggested a possible function for this polymorphism through alteration of TLR-2 expression levels [15]. Thus, it is possible that patients homozygous for the 280-bp allelic length have stronger responses to mycobacterial products and tend to shift their adaptive response toward the Th1 pattern.

Like many genetic-association studies, our study may be affected by population stratification. To address this issue, we performed stratified analysis within each ethnic group. We found that significant associations were consistent among the 3 ethnic groups, suggesting that population stratification may not have been a major issue. As previously mentioned, another limitation results from the fact that the *TLR2* SNPs (such as 597C→T) and/or haplotypes may be in linkage disequilibrium with mutations in another gene located nearby that are responsible for the alterations in the phenotype. In addition, the fact that the multiple end points addressed in this study (susceptibility to leprosy, leprosy type, and leprosy reactions) by use of a multiallelic marker (microsatellite) involves a large number of different tests may lead to false-positive results. There is no definite consensus on which is the best method for achieving multiple-testing correction that will account for the fact that different markers within the same gene are not fully independent from each other [38]. Although the associations between different allelic lengths of the microsatellite marker and leprosy or leprosy type (which had a low level of significance) require validation in a

separate study, the strength and the significance level of the association between the 280-C-T/280-C-T diplotype and reversal reaction were more suggestive of a real genetic effect. Significance would still be achieved if the Bonferroni correction were applied, assuming 100 independent tests. Furthermore, that TLR-2 is clearly involved in the innate immune response to mycobacteria, that mutations in *TLR2* have been shown to be associated with susceptibility to mycobacterial diseases, and that the length of the microsatellite marker influences *TLR2* expression (together with the consistency of the observation among ethnic groups) suggest that the *TLR2* polymorphisms are truly responsible for the observed phenotype.

Overall, our data show that polymorphisms in genes encoding TLRs can, at least in part, explain differences in susceptibility to reversal reaction in leprosy, thereby confirming a role for *TLR2* in the pathogenesis of mycobacterial infections. A larger genetic study may help to refine risk stratification by identifying haplotypes that confer high, intermediate, or low risk of reversal reaction. The present study provides new insights into the immunogenetics of leprosy.

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Table 1

Baseline characteristics of case patients with leprosy and control subjects.

Characteristic	All			Oromo[a]			Amhara[a]			Gurage[a]		
	Leprosy (*n* = 441)	**Control (*n* = 197)**	***P***	**Leprosy (*n* = 193)**	**Control (*n* = 92)**	***P***	**Leprosy (*n* = 126)**	**Control (*n* = 44)**	***P***	**Leprosy (*n* = 112)**	**Control (*n* = 51)**	***P***
Male sex	302 (72)	144 (73)	.70	126 (69)	66 (72)	.68	85 (70)	30 (68)	.85	87 (79)	43 (84)	.52
Age group[b]			<.001			<.001			.009			<.001
0–19 years	41 (10)	43 (22)		20 (11)	21 (23)		15 (13)	12 (29)		3 (3)	9 (18)	
20–39 years	159 (38)	109 (56)		79 (44)	51 (55)		46 (39)	20 (48)		32 (30)	30 (59)	
40–59 years	168 (14)	37 (19)		62 (34)	17 (18)		46 (39)	10 (24)		58 (54)	10 (20)	
≥60 years	45 (11)	5 (3)		19 (11)	3 (3)		11 (9)	0 (0)		15 (14)	2 (4)	
Leprosy type[c]												.47
LL	81 (20)			38 (21)			22 (20)			19 (17)		
BL	199 (48)			92 (51)			58 (52)			47 (43)		
BT	128 (10)			50 (27)			32 (29)			42 (39)		
TT	3 (1)			2 (1)			0 (0)			1 (1)		
Leprosy complication[d]												.85
Reversal reaction	66 (31)			29 (29)			29 (35)			8 (33)		
ENL	17 (8)			7 (7)			9 (11)			1 (4)		
Neuritis	133 (62)			63 (62)			51 (61)			13 (54)		

NOTE. Data are no. (%) of patients, unless otherwise indicated. *P* values are exact *P* values for the overall distribution of sex and age groups within the different ethnic groups and for the overall distribution of leprosy type and leprosy reactions among the 3 different ethnic groups. BL, borderline lepromatous; BT, borderline tuberculoid; ENL, erythema nodosum leprosum; LL, lepromatous lepromatous; TT, tuberculoid tuberculoid.

[a] Ethnicity was missing for 10 patients and 10 control subjects. Thus, totals in the last columns are slightly higher than the totals for case patients from the 3 ethnic groups.

[b] Age was missing for 28 patients and 3 control subjects.

[c] Leprosy type was missing for 5 patients; 25 patients classified according World Health Organization criteria as multibacillary (MB; 18 case patients) or paucibacillary (PB; 7 case patients) are not shown. Therefore, a total of 298 patients were lepromatous (81 LL + 199 BL + 18 MB) and a total of 138 were tuberculoid (128 BT + 3 TT + 7 PB) (see table 4).

[d] Reactions were reported for 216 patients only (see Patients, Materials, and Methods).

Table 2

Allelic frequencies of *TLR2* polymorphisms in the 3 Ethiopian populations.

	Frequency, %			
Marker	**Oromo (*n* = 285)**	**Amhara (*n* = 170)**	**Gurage (*n* = 163)**	***P***
SNP				
597 C/T (rs3804099)	38.7	40.5	37.9	.79
1350 T/C (rs3804100)	4.9	5.4	6.1	.73
Microsatellite[a] allelic length				.87
280 bp	36.2	33.3	34.7	
282 bp	10.6	16.7	14.4	
284 bp	14.7	13.2	13.8	
288 bp	6.9	6.0	6.3	
290 bp	9.9	10.7	10.9	

NOTE. *P* values are for the overall distribution of the polymorphisms among the 3 different ethnic groups. The *P* value for a single-table analysis of all polymorphisms is .67.

[a] Microsatellite indicates the size of the polymerase chain reaction (PCR) product.

J Infect Dis. Author manuscript; available in PMC 2011 April 14.

Table 3

Linkage disequilibrium between the *TLR2* markers in all ethnic groups.

Marker	MS 280 bp	MS 282 bp	MS 284 bp	MS 288 bp	MS 290 bp	SNP 1350T→C	SNP 597C→T
MS 280 bp	**0.33**						
MS 282 bp	0.07	**0.13**					
MS 284 bp	0.06	0.02	**0.13**				
MS 288 bp	0.02	0.01	0.00	**0.06**			
MS 290 bp	0.05	0.01	0.02	0.01	**0.10**		
SNP 1350T→C	0.02	0.01	0.01	0.00	0.00	**0.05**	
SNP 597C→T	0.30	0.03	0.00	0.06	0.18	0.02	**0.39**

NOTE. Off-diagonal elements are estimates of R^2, assuming Hardy-Weinberg equilibrium. Diagonal elements are allele frequencies (in boldface). Pairwise linkage disequilibrium was calculated using the pwld program in Stata (version 9; StataCorp). MS, microsatellite allelic length; SNP, single nucleotide polymorphism.

J Infect Dis. Author manuscript; available in PMC 2011 April 14.

Table 4

Associations between *TLR2* markers and leprosy, leprosy type, and reversal reaction (RR) in all ethnic groups.

	Leprosy status				Leprosy type				RR			
Marker	**Leprosy (*n* = 441)**	**Control (*n* = 197)**	**OR**	***P***	**Lepromatous (*n* = 298)**	**Tuberculoid (*n* = 138)**	**OR**	***P***	**RR (*n* = 66)**	**No RR (*n* = 150)**	**OR**	***P***
SNP												
597C→T												
0/0	38.3	34.7	1.00		39.4	35.9	1.00		48.3	27.3	1.00	
0/1	45.9	51.1	0.78	.24	45.4	46.9	0.91	.69	38.3	56.1	0.31	.003
1/1	15.8	14.2	0.99	.97	15.1	17.2	0.82	.56	13.3	16.5	0.39	.07
1350T→C												
0/0	89.4	89.1	1.00		88.7	90.9	1.00		94.7	89.0	1.00	
0/1	10.3	10.9	1.15	.66	10.9	9.1	1.33	.44	5.3	11.0	0.38	.15
1/1	0.2	0.0			0.4	0.0			0.0	0.0		
Microsatellite[a] allelic length												
280 bp												
0/0	43.0	43.3	1.00		43.0	43.8	1.00		30.5	49.6	1.00	
0/1	43.2	46.0	0.91	.64	43.3	43.1	1.11	.67	47.5	44.7	1.58	.22
1/1	13.8	10.7	1.41	.28	13.7	13.1	1.08	.83	22.0	5.7	7.09	.001
282 bp												
0/0	75.9	72.2	1.00		72.9	81.5	1.00		79.7	72.3	1.00	
0/1	22.7	25.7	0.86	.51	26.1	16.2	1.82	.04	18.6	26.2	0.53	.13
1/1	1.4	2.1	0.76	.71	1.1	2.3	0.49	.39	1.7	1.4	0.96	.97
284 bp												
0/0	73.0	77.5	1.00		73.6	72.3	1.00		74.6	73.8	1.00	
0/1	24.6	19.8	1.15	.56	24.3	24.6	0.87	.58	23.7	24.1	1.02	.97
1/1	2.4	2.7	1.13	.84	2.1	3.1	1.08	.91	1.7	2.1	1.18	.90
288 bp												
0/0	86.2	90.9	1.00		89.1	80.0	1.00		86.4	86.5	1.00	
0/1	13.6	9.1	1.32	.37	10.6	20.0	0.47	.01	13.6	13.5	1.20	.71

Marker	Leprosy status				Leprosy type				RR			
	Leprosy (*n* = 441)	Control (*n* = 197)	OR	*P*	Lepromatous (*n* = 298)	Tuberculoid (*n* = 138)	OR	*P*	RR (*n* = 66)	No RR (*n* = 150)	OR	*P*
1/1	0.2	0.0			0.4	0.0			0.0	0.0		
290 bp												
0/0	82.8	74.9	1.00		83.8	80.8	1.00		79.7	83.0	1.00	
0/1	16.2	23.5	0.63	.05	15.1	18.5	0.85	.57	20.3	15.6	1.27	.58
1/1	1.0	1.6	0.31	.18	1.1	0.8	2.24	.50	0.0	1.4		

NOTE. Listed frequencies (%) are for the presence of 0 (0/0), 1 (0/1), or 2 (1/1) copies of the genotype or allelic length. *P* values were calculated using a logistic regression model including 0/1 and 1/1, with 0/0 as the reference (a general model that did not assume any particular type of inheritance was used), adjusted for age group, sex, and ethnicity. OR, odds ratio.

[a] Microsatellite indicates the size of the polymerase chain reaction (PCR) product.

Table 5

Associations between *TLR2* microsatellite markers and leprosy in the different ethnic groups.

290-bp microsatellite[a] allelic length	All		Oromo		Amhara		Gurage	
	Leprosy (*n* = 441)	Control (*n* = 197)	Leprosy (*n* = 193)	Control (*n* = 92)	Leprosy (*n* = 126)	Control (*n* = 44)	Leprosy (*n* = 112)	Control (*n* = 51)
0/0	82.8	74.9	83.3	76.1	84.3	65.8	80.7	78.4
0/1	16.2	23.5	16.7	21.6	14.0	34.2	17.4	19.6
1/1	1.0	1.6	0.0	2.3	1.7	0.0	1.8	2.0
0/1 + 1/1	17.2	25.1	16.7	23.9	15.7	34.2	19.3	21.6
Additive model								
OR (95% CI)	0.62 (0.41–0.93)		0.66 (0.36–1.21)		0.40 (0.17–0.93)		0.61 (0.25–1.48)	
P	.02		.18		.03		.27	

NOTE. Listed frequencies (%) are for the presence of 0 (0/0), 1 (0/1), or 2 (1/1) copies of the marker. Allele carriage frequencies (0/1 + 1/1) are presented for convenience. Odd ratios (OR) and *P* values were computed under the best-fitting model, which was determined by the likelihood ratio test. The results are adjusted for age group and sex. For the analysis comprising all ethnic groups, results are also adjusted for ethnicity. To account for the additive model, the variable was coded 0, 1, or 2 for the presence of 0, 1, or 2 copies of the allele. CI, confidence interval.

[a]Microsatellite indicates the size of the polymerase chain reaction (PCR) product.

J Infect Dis. Author manuscript; available in PMC 2011 April 14.

Table 6

Associations between *TLR2* markers and leprosy type in the different ethnic groups.

	All		Oromo		Amhara		Gurage	
Marker	**Lepromatous (*n* = 298)**	**Tuberculoid (*n* = 138)**	**Lepromatous (*n* = 137)**	**Tuberculoid (*n* = 53)**	**Lepromatous (*n* = 89)**	**Tuberculoid (*n* = 36)**	**Lepromatous (*n* = 67)**	**Tuberculoid (*n* = 45)**
282-bp microsatellite[a] allelic length								
0/0	72.9	81.5	79.1	83.3	66.7	77.8	68.2	81.4
0/1	26.1	16.2	19.4	16.7	32.1	13.9	31.8	18.6
1/1	1.1	2.3	1.6	0.0	1.2	8.3	0.0	0.0
0/1 + 1/1	27.1	18.5	20.9	16.7	33.3	22.2		
Dominant model[b]								
OR (95% CI)	1.64 (0.96–2.81)		1.22 (0.50–2.99)		1.53 (0.59–3.94)		2.02 (0.75–5.44)	
P	.07		.66		.38		.16	
288-bp microsatellite[a] allelic length								
0/0	89.1	80.0	86.8	75.0	91.7	83.3	89.4	81.4
0/1	10.6	20.0	13.2	25.0	7.1	16.7	10.6	18.6
1/1	0.4	0.0	0.0	0.0	1.2	0.0	0.0	0.0
0/1 + 1/1	10.9	20.0			8.3	16.7		
Dominant model[b]								
OR (95% CI)	0.49 (0.27–0.90)		0.59 (0.24–1.46)		0.41 (0.11–1.45)		0.49 (0.16–1.51)	
P	.02		.25		.16		.22	
Haplotype 288-T-T								
0/0	90.2	81.3	88.8	78.4	92.1	83.3	89.6	81.8
0/1	9.5	18.7	11.2	21.6	6.7	16.7	10.4	18.2
1/1	0.3	0.0	0.0	0.0	1.1	0.0	0.0	0.0
0/1 + 1/1	9.8	18.7			7.9	16.7		
Dominant model[b]								
OR (95% CI)	0.49 (0.26–0.90)		0.60 (0.23–1.54)		0.39 (0.11–1.40)		0.50 (0.16–1.55)	

Marker	All		Oromo		Amhara		Gurage	
	Lepromatous (*n* = 298)	**Tuberculoid (*n* = 138)**	**Lepromatous (*n* = 137)**	**Tuberculoid (*n* = 53)**	**Lepromatous (*n* = 89)**	**Tuberculoid (*n* = 36)**	**Lepromatous (*n* = 67)**	**Tuberculoid (*n* = 45)**
P	.02		.28		.15		.23	

NOTE. Listed frequencies (%) are for the presence of 0 (0/0), 1 (0/1), or 2 (1/1) copies of the marker. Allele carriage frequencies (0/1 + 1/1) are presented for convenience. Odd ratios {OR} and *P* values were computed under the best-fitting model, which was determined by the likelihood ratio test. The results are adjusted for age group and sex. For the analysis comprising all ethnic groups, results are also adjusted for ethnicity. Haplotype nomenclature refers to 3 loci composed of microsatellite 288,597T and 1350T. CI, confidence interval.

[a] Microsatellite indicates the size of the polymerase chain reaction (PCR) product.

[b] (0/1 + 1/1) vs. 0/0.

Table 7

Associations between *TLR2* markers and reversal reaction (RR) in the different ethnic groups.

	All		Oromo		Amhara		Gurage	
Marker	**RR (*n* = 66)**	**No RR (*n* = 150)**	**RR (*n* = 29)**	**No RR (*n* = 72)**	**RR (*n* = 29)**	**No RR (*n* = 54)**	**RR (*n* = 8)**	**No RR (*n* = 16)**
SNP 597C→T								
0/0	48.3	27.3	45.8	24.6	48.3	26.5	57.1	35.7
0/1	38.3	56.1	41.7	53.6	41.4	65.3	14.3	35.7
1/1	13.3	16.5	12.5	21.7	10.3	8.2	28.6	28.6
0/1+1/1	51.7	72.7	54.2	75.4	51.7	73.5	42.9	64.3
Dominant model[a]								
OR (95% CI)	0.34 (0.17–0.68)		0.32 (0.11–0.99)		0.26 (0.09–0.79)		0.19 (0.01–2.99)	
P	.002		.05		.02		.23	
280-bp microsatellite[b] allelic length								
0/0	30.5	49.6	37.5	52.9	25.0	52.0	28.6	37.5
0/1	47.5	44.7	41.7	41.2	53.6	40.0	42.9	62.5
1/1	22.0	5.7	20.8	5.9	21.4	8.0	28.6	0.0
0/1+1/1	69.5	50.4	62.5	47.1	75.0	48.0	71.4	62.5
Recessive model[c]								
OR (95% CI)	5.83 (1.98–17.15)		4.26 (0.84–21.47)		7.51 (1.22–46.37)		…	
P	.001		.08		.03		…	
Haplotype 280-C-T								
0/0	32.3	49.3	40.0	53.5	24.1	48.1	37.5	43.8
0/1	46.8	44.6	40.0	40.8	55.2	42.6	37.5	56.3
1/1	21.0	6.1	20.0	5.6	20.7	9.3	25.0	0.0
0/1+1/1	67.7	50.7	60.0	46.5	75.9	51.9	62.5	56.3
Recessive model[c]								
OR (95% CI)	6.39 (2.14–19.07)		4.55 (0.90–22.90)		10.72 (1.59–72.22)		…	
P	.001		.07		.02		…	

NOTE. Listed frequencies (%) are for the presence of 0 (0/0), 1 (0/1), or 2 (1/1) copies of the marker. Allele carriage frequencies (0/1+1/1) are presented for convenience. Odd ratios (OR) and *P* values were computed under the best-fitting model, which was determined by the likelihood ratio test. The results are adjusted for age group and sex. For the analysis comprising all ethnic groups, results are also adjusted for ethnicity. Haplotype nomenclature refers to 3 loci composed of microsatellites of the indicated lengths, 597C→T and 1350T→C. CI, confidence interval.

[a] (0/1+1/1) vs. 0/0.

[b] Microsatellite indicates the size of the polymerase chain reaction (PCR) product.

[c] 1/1 vs. (0/0+0/1).

J Infect Dis. Author manuscript; available in PMC 2011 April 14.

Table 8

Associations of *TLR2* haplotypes with leprosy, leprosy type, and reversal reaction (RR) in all ethnic groups.

	Leprosy status				Leprosy type				RR			
Haplotype	**Leprosy (*n* = 441)**	**Control (*n* = 197)**	**OR**	***P***	**Lepromatous (*n* = 298)**	**Tuberculoid (*n* = 138)**	**OR**	***P***	**RR (*n* = 66)**	**No RR (*n* = 150)**	**OR**	***P***
280-C-T												
0/0	43.1	42.1	1.00		43.1	44.0	1.00		32.3	49.3	1.00	
0/1	43.8	45.7	0.88	.52	43.4	44.8	1.09	.72	46.8	44.6	1.53	.25
1/1	13.1	12.2	0.99	.97	13.6	11.2	1.10	.79	21.0	6.1	7.63	<.001
290-T-T												
0/0	81.6	75.6	1.00		82.7	79.1	1.00		77.4	82.4	1.00	
0/1	17.5	22.8	0.76	.22	16.3	20.1	0.80	.43	22.6	16.2	1.30	.52
1/1	0.9	1.5	0.33	.20	1.0	0.7	2.19	.52	0.0	1.4		
282-T-T												
0/0	82.0	80.7	1.00		80.7	84.3	1.00		82.3	77.7	1.00	
0/1	17.7	18.8	1.07	.78	19.0	15.7	1.19	.55	17.7	21.6	0.51	.12
1/1	0.2	0.5	1.00	1.00	0.3	0.0			0.0	0.7		
284-C-T												
0/0	82.9	87.3	1.00		83.7	82.1	1.00		82.3	85.8	1.00	
0/1	15.9	12.2	1.28	.37	14.6	17.9	0.81	.49	17.7	13.5	1.56	.32
1/1	1.2	0.5	3.52	.27	1.7	0.0			0.0	0.7		
288-T-T												
0/0	87.3	91.9	1.00		90.2	81.3	1.00		90.3	87.8	1.00	
0/1	12.4	8.1	1.32	.38	9.5	18.7	0.46	.01	9.7	12.2	0.92	.87
1/1	0.2	0.0			0.3	0.0			0.0	0.0		
284-T-T												
0/0	90.1	89.3	1.00		90.8	88.1	1.00		91.9	87.8	1.00	
0/1	9.7	10.7	0.77	.39	8.8	11.9	0.59	.15	8.1	12.2	0.70	.53
1/1	0.2	0.0			0.3	0.0			0.0	0.0		

NOTE. Listed frequencies (%) are for the presence of 0 (0/0), 1 (0/1), or 2 (1/1) copies of the haplotypes. *P* values were calculated using a logistic regression model including 0/1 and 1/1, with 0/0 as the reference (a general model that did not assume any particular type of inheritance was used), adjusted for age group, sex, and ethnicity. Haplotype nomenclature refers to 3 loci composed of microsatellites of the indicated lengths, 597C→T and 1350T→C.