Genetic Variations and Interactions in Anti-inflammatory Cytokine Pathway Genes in the Outcome of Leprosy: A Study Conducted on a MassARRAY Platform

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Background. Mycobacterium leprae is the etiologic pathogen that causes leprosy. The outcome of disease is dependent on the host genetic background.

Methods. We investigated the association of 51 single-nucleotide polymorphisms (SNPs) in anti-inflammatory cytokines (IL-10, TGFB1, IL-6, IL-4, and IL-13) and receptors (IL-10RA, IL-10RB, TGFBR1, TGFBR2, IL-6R, IL-4R, IL-5RA, IL-5RB, and IL-13RA1) with susceptibility to leprosy in a case-control study from New Delhi in northern India. This was followed by replication testing of associated SNPs in a geographically distinct and unrelated population from Orissa in eastern India. The functional potential of SNPs was established with in vitro reporter assays.

Results. Significant associations (P < .05) were observed for 8 polymorphisms (rs1800871, rs1800872, and rs1554286 of IL-10; rs3171425 and rs7281762 of IL-10RB; rs2228048 and rs744751 of TGFBR2; and rs1800797 of IL-6) with leprosy. This association was replicated for 4 SNPs (rs1554286 of IL-10, rs7281762 of IL-10RB, rs2228048 of TGFBR2, and rs1800797 of IL-6). The interaction study revealed a significantly greater association with leprosy risk than was obtained for any SNP individually.

Conclusions. This study provides an interesting insight on the cumulative polygenic host component that regulates leprosy pathogenesis.

Leprosy is a chronic granulomatous infection due to the intracellular organism Mycobacterium leprae, which is known to affect an estimated 211,903 new patients annually [1]. It is characterized by clinically defined polar manifestations that depend upon the host cell–mediated immune response against the pathogen. Tuberculoid and lepromatous leprosy are at opposite ends of the spectrum, associated either with a strong cell-mediated immunity and T helper 1 (Th1) cytokine profile or with T helper 2 (Th2) cytokine and strong humoral responses, respectively [2]. The limited genetic diversity between M. leprae isolates [3] and the clinical spectrum observed among individuals who develop leprosy illustrates the significance of host factors in influencing the outcome of the infection. Evidence from familial clustering, segregation, twin studies [4–6], and recent genome-wide studies [7, 8] suggests that host genetic factors might contribute to the interindividual variability. Several host genes that modulate immune response to M. leprae infection have been suggested to influence the acquisition of leprosy as well as its clinical course [2, 9]. Among these, TLR1, PARK2/PACRG, LTA, and TAP have been identified in numerous case-control studies.
MATERIALS AND METHODS

Subjects
A total of 2447 samples were studied (obtained from 734 patients from northern India; 73 patients from Orissa, in eastern India; 1294 unrelated healthy control subjects from northern India; and 346 unrelated healthy control subjects from Orissa, eastern India). Northern Indian study subjects were recruited at Lok Nayak Jai Prakash Hospital, New Delhi, and at Guru Teg Bahadur Hospital, Delhi, whereas the study subjects from eastern India were recruited at Cuttack Leprosy Home and Hospital, Orissa. Diagnosis of leprosy was made by at least 2 independent leprologists after a physical examination of each patient and standard histological and pathological examination of affected skin lesions [27]. All patients were under treatment with multidrug therapy (MDT) specific for multibacillary (MB) and paucibacillary (PB) leprosy, as recommended by the World Health Organization. None of the control subjects had any family history of tuberculosis, leprosy, diabetes, or any other related diseases (see Supplementary data). An informed written consent following the Indian Council of Medical Research norms was obtained from all individuals whose blood samples were collected. The study was approved by the Jawaharlal Nehru University ethics committee.

SNP Selection
To explore the role of anti-inflammatory cytokines, we selected a group of important cytokine genes (IL-10, TGFB1, IL-6, IL-4, and IL-13) and their receptor chains (IL-10RA, IL-10RB, TGFB1R, TGFB2R, IL6R, IL-4R, IL-5RA, IL-5RB, and IL-13RA1) to determine the causal functional variants for leprosy in the studied population groups. SNPs were selected by prioritization based on their minor allele frequency (>5%) in the publicly available dataset from the National Centre for Biotechnology Information (NCBI) EntrezSNP (build 36) and the International HapMap project: Han Chinese, Japanese (Asian populations), and African (Ancestral) populations. Few of the SNPs were included either on the basis of functional role demonstrated or putative and documented in literature or on their presence in promoter, exonic, intronic boundary, or untranslated regions (UTR) covering 2 kb upstream and downstream of each gene. The sequence for all SNPs was downloaded from the Chip Bioinformatics database [28], and the sequences were cross-checked in NCBI before assay design.

Genotyping
High-throughput genotyping of SNPs was performed using IplexTM GOLD chemistry on a matrix-assisted laser desorption, ionization time-of-flight mass spectrometer (Sequenom). SNPs with a call rate <90% were removed. Significant SNPs had a call rate >95%. The individual call rate threshold was at least 95% (see Supplementary data).

Vector Construction
Luciferase reporter plasmids were constructed using the pGL3 Vector (Promega) carrying a SV40 promoter and luciferase expression unit. Mutated versions of these constructs were generated by site-directed mutagenesis. All mutations were verified by sequencing (see Supplementary data for details).

Transient Transfection and Luciferase Assays
For the luciferase reporter assay, HeLa (human cervical cancer), HEK293 (normal human embryonic kidney), and HepG2 (human hepatocellular liver carcinoma cell line) cell lines were used (see Supplementary data for details).

Statistical Analysis
Statistical analyses of the polymorphic variant frequencies of candidate genes were performed in a stepwise manner. First,
overall genotype and allele frequencies of patients and control subjects were compared using a 3 × 2 and 2 × 2 χ² test; if a significant overall difference was detected (P < .05), then individual SNP genotypes were compared using a logistic regression with adjustment for sex to calculate odds ratios (ORs) and 95% confidence intervals (CIs). Logistic regression was also used to determine the interactive association between the genotypic status of the studied polymorphisms after correction with sex. SPSS software, version 17 (SPSS), was used for statistical analysis. The genotype frequencies for SNPs were subjected to Hardy-Weinberg equilibrium (HWE) analysis in combined samples (case patient and control subject), and SNPs with deviations (P < .01) from HWE were removed as a quality control criterion. For the multiple comparisons, the false discovery rate [29] estimation method was applied to the association analyses by using Golden Helix SVS 7.

Linkage disequilibrium structure was determined using Haploview software, version 4.2 [30]. Haplotype frequencies were estimated by using HaploStats software (R, version 1.4.0), which implements an expectation-maximization algorithm. To ensure adequate statistical results with an association study, power calculation was calculated in Quanto [31]. Power was defined as the probability correctly rejecting the null hypothesis that the OR was equal between case patients and control subjects. Power in the association study was dependent on several factors: allele frequency, the type I error (false positive) rate, the OR, and the sample size. Power was calculated for the association study in combined samples from Delhi and Orissa based on allele frequency and the effect size of the respective polymorphism.

Plink, version 1.06, was used for multidimensional scaling (MDS) for population stratification. For population differentiation analysis, Fst was calculated by the formula \( F_{ST} = (H_T - H_S)/H_T \), where \( H_S \) and \( H_T \) are the global heterozygosity indices over subpopulations (patients, control subjects, and 4 HapMap populations) and total population.

**RESULTS**

A population-based case-control study was performed for SNPs within anti-inflammatory cytokine and receptor genes in a population from northern India. To rule out population stratification, which confounds a disease association study, an MDS analysis generated a compact cluster that indicated a population of patients and control subjects to be homogenous with no substructures (Figure 1). The same conclusion was drawn using a set of representative samples from our cohort in a genome-wide analysis [8]. MDS is robust for a large number of SNPs; therefore, the locus-wise \( F_{ST} \) was also calculated between patients and control subjects for SNPs that were found to be associated with leprosy in samples from New Delhi. All polymorphisms showed a very low locus-wise \( F_{ST} \) value. The results indicated that the studied SNPs did not undergo population differentiation and that our patients and control subjects belonged to the same population group. The details of distribution, allele frequencies, and HWE \( P \) values for the studied SNPs are provided in Supplementary Table 1. A total of 8 SNPs, located in the **IL-10**, **IL-10RB**, **TGFBR2**, and **IL-6** genes, showed a strong significant association with susceptibility to leprosy (Table 1) in the northern Indian population.

An earlier preliminary study from our laboratory involving the **IL-10** gene showed an involvement of most of the functionally important SNPs in the promoter region [18]. It was pertinent to find out whether any other functionally important SNP within the gene could play an additional role in determining susceptibility to leprosy. Four SNPs were chosen for such a study: rs1554286, which is located at the intronic boundary of intron 3, creating a putative location of an alternate splicing, and rs3024496, rs3024498, and rs3024500 at 3’ UTR. Of these 4 variants, rs1554286 showed a significant risk towards leprosy per se (TT/CT+CC; OR, 1.66; 95% CI, 1.30–2.14; \( P < .001 \)). Among the promoter polymorphisms, rs1800872 (−592CA) and rs1800871 (−819C/T) appeared to provide protection against leprosy per se (CC/CA+AA [OR, 0.76; 95% CI, .62–.92; \( P = .006 \)] and CC/CT+TT [OR, 0.75; 95% CI, .62–.92; \( P = .005 \)], respectively).

Next, we identified 2 SNPs within **IL-10RB** to be significantly associated with the disease; one, rs3171425, was located in the 3’ UTR (GG/GA+AA; OR, 1.36; 95% CI, 1.13–1.64; \( P = .001 \)) and the other, rs7281762, was located downstream of the 3’ UTR (GA+AA/GG; OR, 1.33; 95% CI, 1.1–1.6; \( P = .002 \)). None of the variants of **IL-10RA** were found to be significantly associated with leprosy.

Furthermore, 2 polymorphisms (synonymous variant rs2228048 [CC/CT+TT; OR, 0.77; 95% CI, 0.63–0.95; \( P = .015 \)] and rs744731, located downstream of the 3’ UTR [GG/GA+AA; OR, 1.32; 95% CI, 1.1–1.62; \( P = .006 \)] within **TGFBR2** showed significant association with leprosy. Another promoter polymorphism, rs1800797 of **IL-6**, was also observed to be significantly associated with leprosy (GG/GA+AA; OR, 1.33; 95% CI, 1.1–1.64; \( P = .008 \)).

An analysis after delineating the patients in 2 polar forms of the disease (PB and MB) showed 5 of 8 SNPs in strong association with the MB polar group, compared with the PB polar group, whereas the 2 **IL-10** promoter SNPs and 1 **IL-6** were in strong association with the PB polar group (see Supplementary data).

**Replication Analysis**

We sought to replicate these associations in a geographically unrelated Indian population of 491 individuals from Orissa in eastern India, and we genotyped 8 SNPs (3 of **IL-10**, 2 of **IL-10RB**, 2 of **TGFBR2**, and 1 of **IL-6**), which was found to be significant in studied samples from northern India (Table 1).

The replicative analysis revealed significant evidence of an association at 4 SNPs in the Orissa population. These included...
rs1554286 of *IL-10* (TT/CT+CC; OR, 1.98; 95% CI, 1.13–3.50; \( P = .016 \)) and rs7281762 of *IL-10RB* (GA+AA/GG; OR, 2.51; 95% CI, 1.42–4.42; \( P = .002 \)); however, the association was more significant for GA+GG/AA; rs2228048 within *TGFBR2* (CC/CT+TT; OR, 0.52; 95% CI, .31–.87; \( P = .013 \)) and rs1800797 in *IL-6* (GG/GA+AA; OR, 4.9; 95% CI, 1.50–16.05; \( P = .01 \)).

Furthermore, a combined analysis of northern and eastern Indian samples for these SNPs confirmed the strong association for the 4 SNPs that were replicated in the Orissa population as well as for the rest of the SNPs: rs1554286 of *IL-10* (TT/CT+CC; OR, 1.55; 95% CI, 1.24–1.95; \( P < .001 \)); rs7281762 of *IL-10RB* (GA+AA/GG; OR, 1.31; 95% CI, 1.11–1.56; \( P = .002 \)); rs2228048 within *TGFBR2* (CC/CT+TT; OR, 0.78; 95% CI, .64–.94; \( P = .01 \)); rs1800797 in *IL-6* (GG/GA+AA; OR, 1.31; 95% CI, 1.10–1.60; \( P = .01 \)); rs1800871 in *IL-10* (CC/CT+TT; OR, 0.79; 95% CI, .66–.96; \( P = .014 \)); rs1800872 in *IL-10* (CC/CA+AA; OR, 0.78; 95% CI, .65–.95; \( P = .012 \)); rs3171425 of *IL-10RB* (GG/GA+AA; OR, 1.30; 95% CI, 1.10–1.52; \( P = .004 \)) and rs744751 within *TGFBR2* (GG/GA+AA; OR, 1.30; 95% CI, 1.05–1.53; \( P = .012 \)), even after adjustment with sex as a covariate. The associations at rs1554286, rs3171425, rs7281762, rs2228048, and rs1800797 remained statistically significant after false discovery rate correction. Two promoter SNPs of *IL-10*, rs1800871 (\( P = .05 \)) and rs1800872 (\( P = .048 \)) were marginally significant after false discovery rate analysis (Table 1).

A power calculation in combined samples from northern and eastern India with effect size and allele frequency of respective SNPs indicated the following: rs1554286 had >90%; rs7281762, rs744751, rs1800871, rs2228048, rs1800797, and rs3171425 had 80%–89%; and rs1800872 had 76% power to detect an association with the disease.

Furthermore, a linkage disequilibrium (LD) analysis was performed for *IL-10, IL-10RB, TGFBR2,* and *IL-6,* because the variants on these genes were found to be significantly associated with the disease in combined analysis. A strong LD (\( r^2 > 0.8 \)) was observed among 3 *IL-10* SNPs: rs1800871, rs1800872, and rs1554286. These 3 markers were making a haplotype bin, and any of these markers could be defined as tag (Figure 2A).

The pairwise LD analysis between markers of *IL-10RB* showed weak LD (\( r^2 < 0.15 \)) between rs3171425 and rs7281762 (Figure 2B). Therefore, both SNPs contributed independently toward leprosy susceptibility. None of the SNPs were in LD in *TGFBR2* and *IL-6* (Figure 2C and D).

**Interaction Analysis**

Genotype interaction analysis was performed between significantly associated variations of functionally relevant candidates, such as *IL-10-IL-10RB* and *IL-10-IL-6,* in combined samples from Delhi and Orissa. Because 3 SNPs in *IL-10* were in perfect LD, only 1 SNP, rs1554286, was randomly selected for interaction analysis.
Table 1. Association Statistics of Single-Nucleotide Polymorphisms (SNPs) Within 4 Candidate Genes, IL-10, IL-10RB, TGFBR2, and IL-6, in Different Cohorts of Patients with Leprosy

<table>
<thead>
<tr>
<th>SNP</th>
<th>Delhi Patients</th>
<th>Delhi Controls</th>
<th>Orissa Patients</th>
<th>Orissa Controls</th>
<th>Combined Patients</th>
<th>Combined Controls</th>
<th>P Value</th>
<th>Odds Ratio (95% CI)</th>
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<tbody>
<tr>
<td>rs1800871</td>
<td>213 (29.1)</td>
<td>463 (35.8)</td>
<td>10 (13.7)</td>
<td>223 (27.6)</td>
<td>540 (22.9)</td>
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<tr>
<td>rs1800872</td>
<td>213 (29)</td>
<td>461 (35.6)</td>
<td>10 (13.7)</td>
<td>82 (23.7)</td>
<td>223 (27.6)</td>
<td>543 (33.1)</td>
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<tr>
<td>rs1554286</td>
<td>250 (34.1)</td>
<td>527 (40.7)</td>
<td>11 (15.1)</td>
<td>99 (28.6)</td>
<td>261 (32.3)</td>
<td>626 (38.2)</td>
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<tr>
<td>rs3171425</td>
<td>388 (52.9)</td>
<td>588 (45.4)</td>
<td>194 (56.1)</td>
<td>434 (53.8)</td>
<td>782 (47.7)</td>
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<td>&lt;.001</td>
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<tr>
<td>rs7281762</td>
<td>374 (51.0)</td>
<td>753 (66.5)</td>
<td>19 (26)</td>
<td>158 (45.7)</td>
<td>393 (48.7)</td>
<td>911 (55.5)</td>
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<tr>
<td>rs744751</td>
<td>534 (72.8)</td>
<td>861 (66.5)</td>
<td>51 (69.9)</td>
<td>243 (70.2)</td>
<td>585 (72.5)</td>
<td>1104 (67.3)</td>
<td>&lt;.001</td>
<td>1.31 (1.11–1.53)</td>
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<tr>
<td>rs1800797</td>
<td>566 (77.1)</td>
<td>927 (71.6)</td>
<td>70 (95.9)</td>
<td>287 (82.9)</td>
<td>636 (78.8)</td>
<td>1214 (74)</td>
<td>&lt;.001</td>
<td>1.31 (1.10–1.60)</td>
</tr>
</tbody>
</table>

Data are no. (%) of subjects, unless otherwise indicated.
Abbreviations: FDR, false discovery rate; OR, odds ratio.

* P value for comparison of frequencies by logistic regression analysis between total patients with leprosy and control subjects (sex adjusted) in samples from Delhi, samples from Orissa, and combined samples.

b P values after FDR correction.

* OR for the significant adjusted P values obtained by comparison of frequencies between patients and control subjects in combined samples by logistic regression analysis.
Figure 2. Haplotype analysis of the studied single-nucleotide polymorphisms located in IL-10 (A), IL-10RB (B), TGFBR2 (C), and IL-6 (D), expressed as $-\log_{10} P$ ($P$ value) and linkage disequilibrium plot based on pairwise linkage disequilibrium ($r^2$).
Table 2. Interaction between the IL-10 and IL-10RB Polymorphism in Combined Leprosy Samples

<table>
<thead>
<tr>
<th>rs1554286</th>
<th>rs7281762</th>
<th>Patients</th>
<th>Control subjects</th>
<th>P*</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT+CC</td>
<td>GG</td>
<td>327 (40.5)</td>
<td>795 (48.5)</td>
<td>1 (referent)</td>
<td></td>
</tr>
<tr>
<td>CT+CC</td>
<td>GA+AA</td>
<td>320 (39.7)</td>
<td>620 (37.8)</td>
<td>.02</td>
<td>1.25 (1.03–1.51)</td>
</tr>
<tr>
<td>TT</td>
<td>GG</td>
<td>66 (8.2)</td>
<td>116 (7.1)</td>
<td>.064</td>
<td>1.40 (0.98–1.88)</td>
</tr>
<tr>
<td>TT</td>
<td>GA+AA</td>
<td>94 (11.6)</td>
<td>109 (6.6)</td>
<td>&lt;.001</td>
<td>2.12 (1.56–2.85)</td>
</tr>
</tbody>
</table>

Data are no. (%) of subjects, unless otherwise indicated.
Abbreviations: CI, confidence interval; OR, odds ratio.
* P values after sex adjustment.

To test the enhancer activity of the SNP within IL-10RB, its downstream region was cloned in pGL3 control vector in 3 allele combinations, because these are represented in the studied population of New Delhi, in northern India: clone1 with rs3171425(G)-rs7281762(G), representing the risk allele for rs3171425; clone2 with rs3171425(A)-rs7281762(G), representing the protective allele combination; and clone 3 with rs3171425(G)-rs7281762(A), representing the risk allele combination for both SNPs. A significant overexpression of luciferase gene was observed for clone 3 (G-A combination) (Figure 3A–C) and clone 2 (A-G combination) in all 3 cell lines, compared with clone 1 (G-G combination) (Figure 3D–F).

In IL-6 promoter SNP rs1800797, the expression level for A allele that provided protection was significantly up-regulated, compared with that of G, in HEK (P = .036) and HeLa (P = .043) (Figure 3G–I). We concluded that the A allele of IL-6 promoter, which is overrepresented in control subjects, could up-regulate IL-6 expression.

In TGFBR2, the risk allele (C) of rs744751 significantly reduced the luciferase expression in HeLa, HEK, and HepG2 cell lines, which indicated that the C allele, overrepresented in patients, could down-regulate TGFBR2 gene expression (Figure 3J–L).

**DISCUSSION**

Despite the widespread implementation of multidrug therapy for leprosy, the worldwide incidence of leprosy has shown little decrease. This indicates that an important aspect of the intricate relationship between M. leprae and its host is not yet understood. Genome-wide and candidate gene analysis has

Table 3. Interaction Between the IL-10 and IL-6 Polymorphism in Combined Leprosy Samples

<table>
<thead>
<tr>
<th>rs1554286</th>
<th>rs1800797</th>
<th>Patients</th>
<th>Control subjects</th>
<th>P*</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT+CC</td>
<td>GG</td>
<td>503 (62.3)</td>
<td>1052 (64.1)</td>
<td>1 (referent)</td>
<td></td>
</tr>
<tr>
<td>CT+CC</td>
<td>GA+AA</td>
<td>144 (17.8)</td>
<td>363 (22.1)</td>
<td>.105</td>
<td>0.83 (0.66–1.04)</td>
</tr>
<tr>
<td>TT</td>
<td>GG</td>
<td>133 (16.5)</td>
<td>162 (9.9)</td>
<td>&lt;.001</td>
<td>1.66 (1.34–2.22)</td>
</tr>
<tr>
<td>TT</td>
<td>GA+AA</td>
<td>27 (3.3)</td>
<td>63 (3.8)</td>
<td>.6</td>
<td>0.88 (0.55–1.41)</td>
</tr>
</tbody>
</table>

Data are no. (%) of subjects, unless otherwise indicated.
Abbreviations: CI, confidence interval; OR, odds ratio.
* P values after sex adjustment.
implicated several immunogenetic loci as risk factors for susceptibility to leprosy. However, many of these associations are not replicated in unrelated population groups. In addition, the functional roles of only a few implicated loci are known in leprosy pathogenesis. Recently, in a genome-wide association study involving a Chinese population, a consistent association with leprosy was observed for \textit{CCDC122}, \textit{C13orf31}, \textit{NOD2}, \textit{TNFSF15}, \textit{HLA-DR}, and \textit{RIPK2} [7]. However, these findings could not be replicated in the Indian population [35]. The present study represents a pathway-based approach involving the anti-inflammatory cytokine network directed towards investigating the role of functional genetic variants alone or in combination in a case-control study. Eight genetic variants (rs1800871, rs1800872, and rs1554286 of \textit{IL-10}; rs3171425 and rs7281762 of \textit{IL-10RB}; rs2228048 and rs744751 of \textit{TGFBR2}; and rs1800797 of \textit{IL-6}) were observed to be significantly associated with leprosy in a combined analysis. However, rs744751 did not pass false discovery rate correction for multiple comparisons. We used the false discovery rate method and replicated our results in independent samples [36] to eliminate false-positive associations. We propose that polymorphisms of anti-inflammatory candidates, such as \textit{IL-10}, \textit{IL-10RB}, and \textit{IL-6},
functionally regulate their expression and increase either protection or risk when studied in combination. In addition, the present study convincingly reflects the role of IL-10 promoter variants in leprosy susceptibility, as suggested in our preliminary study, in which a pronounced effect of proximal promoter SNPs (−1082A>G, −819C>T, −592C>A) was observed in determining risk and protection, compared with that of distal SNPs (−3575T>A, −2849G>A, −2763C>G) [18]. In this study, as well, we observed that rs1800871 (−819C>T) and rs1800872 (−592C>A), the promoter SNPs, regulate the expression of IL-10 [32, 33] and show an association with leprosy. Furthermore, SNP rs1554286 of IL-10 showed the strongest association with leprosy among all of the associated variants in the samples from northern India and was in complete LD with the other 2 promoter SNPs. Interestingly, IL-10RB is located in a class II cytokine receptor gene cluster together with interferon (IFN) α receptor 1 (IFNAR1) and 2 (IFNAR2) and IFN-γ receptor 2 (IFNGR2) on chromosome 21q22 [37]. It is likely that SNPs of IL-10RB, either independently or possibly in LD with unknown functional SNPs in any of the IFN receptors, show the effect of IL-10RB in disease progression. This would require a high-resolution scan of the class II cytokine receptor gene cluster in the future. The SNP rs7281762 of IL-10RB also showed a significant association with tuberculosis, which is an observation that is, to our knowledge, made for the first time (unpublished data). Incidentally, both the mycobacterial species have an overlapping antigenic repertoire, and the diseases share immunological features.

The importance of the role of IL-10 promoter SNP and the IL-10RB variations in disease susceptibility was reflected further in the interaction analysis. The concurrent presence of genotypes rs1554286 TT and rs7281762 GA+AA provided highly significant risk of leprosy. Haplotype analysis of SNP alleles showed that the rs1554286 T allele represented the ATA haplotype of promoter SNPs (rs1800871, rs1800872, and rs1800896) (see Supplementary data), and this haplotype was shown to down-regulate the IL-10 expression [32]. Because the A allele of rs7281762 is associated with increased IL-10RB expression, we propose that an increased expression of IL-10RB may enhance STAT3 activation and IL-10 production [38], leading to immunosuppression and the down-regulation of Th1 response and, consequently, of the protective immune response. The observations made here provide evidence for the possibility of IL-10 and its modulation by other gene products along the pathway to affect the final outcome of the response towards an infecting pathogen.

The importance of the synonymous SNP rs2228048 of TGFBR2, which is significantly associated with leprosy in the samples from both New Delhi and Orissa, is not clear. Nevertheless, supporting evidence of rs2228048 (Asn389Asn) as a causal variant in a genetic study was found to be most significantly associated with chronic kidney disease in individuals at low risk among a cohort 2828 Japanese individuals [39].

A multifunctional cytokine, IL-6, has suppressive effects on macrophages, astrocytes, and fibroblasts and suppresses the expression of IL-12, IFN-γ, tumor necrosis factor α, adhesion molecules, and proteases both in vitro and in vivo [40]. The overall role of IL-6 in a particular inflammatory process is determined by the balance between its pro- and anti-inflammatory actions on different cell types. It was surprising that the interaction between rs1554286 TT of IL-10, which is associated with low IL-10 production [32], and the low IL-6–producing −597G allele of rs1800797 provided a significant risk of leprosy. However, the outcome of mycobacterial infection involves a complex interaction between several host genes, and it is difficult to make a conjecture as to the role of receptor-ligand interaction of IL-10 and IL-10RB and the modulation of IL-10 expression in the genetic background of other pro- and anti-inflammatory genes in leprosy susceptibility. Nevertheless, the study reveals the role of multigenic interaction in providing graded risk of or protection against leprosy.

In conclusion, this study suggests that anti-inflammatory cytokine and receptor genes are important genetic determinants of susceptibility to leprosy. It will be interesting to investigate the effect of these polymorphisms on cytokine production in patients and control subjects with defined genotype backgrounds. Furthermore, proinflammatory cytokines and other relevant genes and regions should also be determined to better understand the interactive role of host genetic factors in the etiology of leprosy for efficient interventions.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://www.oxfordjournals.org/our_journals/jid/).

Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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