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LARGE-SCALE CANDIDATE GENE STUDY OF LEPROSY SUSCEPTIBILITY IN THE KARONGA DISTRICT OF NORTHERN MALAWI

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Abstract. We present a large case-control candidate gene study of leprosy susceptibility. Thirty-eight polymorphic sites from 13 genes were investigated for their role in susceptibility to leprosy by comparing 270 cases with 452 controls in Karonga district, northern Malawi. Homozygotes for a silent T→C change in codon 352 of the vitamin D receptor gene appeared to be at high risk (odds ratio [OR] = 4.3, 95% confidence interval [CI] = 1.6–11.4, $P = 0.004$), while homozygotes for the McCoy b blood group defining variant K1590E in exon 29 of the complement receptor 1 (formerly CD35) gene appeared to be protected (OR = 0.3, 95% CI = 0.1–0.8, $P = 0.02$). Borderline evidence for association with leprosy susceptibility was found for seven polymorphic sites in an additional six genes. Some of these apparent associations may be false-positive results from multiple comparisons, and several associations suggested by studies in other populations were not replicated here. These data provide evidence of inter-population heterogeneity in leprosy susceptibility.

INTRODUCTION

Leprosy is a chronic disabling disease attributable to infection with *Mycobacterium leprae.* It has a broad clinical spectrum: at one pole is paucibacillary (PB) leprosy, characterized by a low bacterial count, strong cell-mediated immunity, and localized disease. At the other pole, multibacillary (MB) leprosy is characterized by a high bacterial count, poor cell-mediated immunity and strong humoral immunity, with progressive and disseminated disease. The natural history of leprosy is poorly understood, but family studies, segregation analyses, and twin studies provide evidence that, in addition to environmental and exposure factors, host genetic factors influence leprosy susceptibility.[1–4]

Elucidation of genetic determinants of host susceptibility to leprosy could facilitate the development of better preventative and therapeutic strategies. The earliest strong evidence pointed towards the major histocompatibility complex region on chromosome 6.[5,6] More recently susceptibility loci have been mapped using linkage analysis of multi-case families to chromosomes 10p13 and 20p12 in populations in south India and to chromosome 6q25 and, again, 10p13, in Vietnam.[4,7,8] The linkage on chromosome 10p13 appears to be mainly with tuberculoid leprosy and the chromosome 6q25 linkage with leprosy *per se*. Association studies in various populations have indicated that several other non-HLA genes may also influence either leprosy susceptibility or the type of leprosy that develops upon infection.[3] In general, these association studies have been carried out on relatively small sample sizes, and some of the reported associations may represent chance findings, particularly in studies that examined numerous variants of multiple genes. A major objective of the present study was to re-evaluate reported associations in an independent population.

The Karonga Prevention Study (KPS), a long-term epidemiologic study of mycobacterial disease in the Karonga District of northern Malawi, provides an excellent opportunity to attempt to replicate previously reported leprosy associations as well as to investigate *de novo* hypotheses. A case-control association study was thus designed to investigate a large number of putative susceptibility genes (38 polymorphisms from 13 genes) in this population. The genotyping was performed blind to the disease status of the samples.

Polymorphisms in tumor necrosis factor (TNF, formerly TNFα), the vitamin D receptor (VDR), toll-like receptor 2 (TLR2), and lymphotoxin alpha (LTA, formerly TNFβ) are associated with leprosy in other populations and were included here in an attempt to replicate these findings.[3] An interleukin-10 (IL10) promoter that has recently been suggested to be associated with leprosy susceptibility in Brazil, and other polymorphisms that may influence expression of the immunomodulatory genes IL10 and TLR2 were included (Segal S and others, unpublished data).[9] Polymorphisms in the genes encoding solute carrier family 11, member 1 (SLC11A1, formerly NRAMP1), mannose-binding lectin (MBL2, formerly MBP or MBL) and interferon-γ (IFNG) were investigated because they have been reported to be associated, in at least one population, with susceptibility to a related mycobacterial disease, tuberculosis.[10] The toll-like receptor 4 (TLR4), IL10, and intercellular adhesion molecule 1 (ICAM-1, formerly CD54) loci contain polymorphisms that are associated with susceptibility to other infectious diseases, and it was speculated that these genes might represent general infectious disease susceptibility loci.[11–13] The caspase recruitment domain family, member 15 (CARD15, formerly NOD2) and CR1 were selected as functional candidates because of their roles in innate immunity and potential role in mycobacterial disease. The chemokine (C-C motif) ligand 3 (CCL3, formerly MIP1α) was selected as a functional candidate due to its involvement in T helper cell 1 (TH1) differentiation.

METHODS

The study protocol was approved by the National Health Sciences Research Committee of Malawi and by the Ethics Committee of the London School of Hygiene and Tropical Medicine.

Recruitment and sample collection. Cases were ascertained within the context of the KPS, a long-term epidemiologic study of mycobacterial disease in the Karonga District, a rural area of northern Malawi. The population and basic

field methods of the KPS have been described in detail elsewhere.[14,15] Leprosy cases were identified in the context of total population surveys during the 1980s, and through "enhanced" passive detection (examination of all individuals who appeared at health centers) in the 1990s, in addition to self-reporting. Individuals were examined first by paramedical leprosy control assistants (LCAs), and suspects referred to project Medical Officers for review examination and (4-mm punch) biopsy. Diagnostic certainty was defined in terms of clinical, skin slit smear, and histopathologic data.[16] Cases were defined as PB or MB based upon an algorithm that took into account all clinical, bacteriologic (slit skin smear), biopsy, and historical record information. The algorithm implies a conservative definition of MB (e.g., biopsy or slit skin smear bacterial index > 1).

Recruitment for this study began in 1996. Since leprosy incidence had decreased dramatically in the district, cases were selected retrospectively from project data files. Only cases that met strict diagnostic criteria were included (N and M categories as stipulated elsewhere[16]). Individuals were eligible for selection as controls if they were resident in the district during the 1986–1989 population survey, and had no history of either tuberculosis or leprosy. Controls were matched to cases by age (15–19, 20–24, 25–29, 30–34, 35–44, 45–54, 55–64, and > 65 years old), sex, and geographic area (residence within 1 km of the case). First- or second-degree relatives of cases were not eligible for selection as controls. Each case was matched to up to three controls.

Cases and controls were visited in their homes by field teams consisting of an LCA and an interviewer. After having the study explained to them, those who consented were examined and invited to provide a blood or saliva sample for genetic testing (those who were willing to provide blood were told their blood group, and offered syphilis tests with treatment and also human immunodeficiency virus [HIV] counseling and testing if they so desired). All cases ascertained since 1987 have been offered voluntary counseling and HIV testing at the time of initial registration as part of a continuous case control study of leprosy, tuberculosis, and HIV.[17] Blood (7.5 mL) was collected into EDTA and placed in vaccine carriers with wet ice until it reached the project laboratory within three days of collection, where cells were separated and stored at –70°C. The DNA was separated using Nucleon kits (Scotlab, Coatbridge, United Kingdom) following the manufacturer's instructions, and shipped at 4°C to the Wellcome Trust Center for Human Genetics in Oxford, United Kingdom for analysis.

Genotyping. Genotyping was performed blind to disease status. A variety of genotyping methods were used to investigate 38 polymorphisms from 13 candidate genes (Tables 1 and 2). Four CARD15 polymorphisms were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), the IFN-γ +874 single nucleotide polymorphism (SNP) was typed using amplification refractory mutation system PCR, and the CARD15 and IFNG products were analyzed using agarose gel electrophoresis.[18,19] Seven polymorphisms in SLC11A1, LTA, CCL3, and TLR2 were genotyped by fluorescence PCR followed by electrophoresis and detection using an ABI fluorescent DNA sequencer (Applied Biosystems, Foster City, CA) as de-

TABLE 1
Oligonucleotides and conditions used for non-LDR genotyping*

Gene	Location, polymorphism Amino acid change (Restriction enzyme)	Genotyping method (% agarose gel)	PCR primers	PCR temperature product length
CARD15 (NOD2)	Exon 4, 802C→T P268S (*Bam* HI)	PCR-RFLP (3%)	F: CAGTCTCGCTTCCTCAGTACC R: AGTGTCCGCATCGTCATTG	55°C 187 bp
	Exon 4, 2104C→T R702W (*Msp* I)	PCR-RFLP (3%)	F: TTCCTGGCAGGGCTGTTGTCCtGG R: GGATGGAGTGGAAGTGCTTG	55°C 139 bp
	Exon 8, 2722G→C G908R (*Hha* I)	PCR-RFLP (3%)	F: AGGCCACTCTGGGATTGAG R: GTGATCACCCAAGGCTTCAG	55°C 196 bp
	Exon 11, 3020insC 1007fs (*Apa* I)	PCR-RFLP (4%)	F: GGCAGAAGCCCTCCTGCAGGgCC R: CCTCAAAATTCTGCCATTCC	55°C 152 bp
CCL3 (MIP1-α)	Promoter, microsatellite $(TA)_n$ at –906	Fluorescence PCR	F: FAM-AAGGCATGTATTTCCAAGC R: CTGACCCAGCATCGTTTA	58°C 327–337 bp
IFNG	Intron 1 874T→A	ARMS PCR (2%)	IFNGT: TTCTTACAACACAAAATCAAATCT IFNGA: TTCTTACAACACAAAATCAAATCA IFNGCOM: TCAACAAAGCTGATACTCCA HGHF: GCCTTCCCAACCATTCCCTTA HGHR: TCACGGATTTCTGTTGTGTTTC	62°C, 50°C 461 bp 426 bp
LTA (TNF-β)	5′UTR, microsatellite $(AC/GT)_n$ at –3.5 kb	Fluorescence PCR	F: GCCTCTAGATTTCATCCAGCCACAG R: HEX-CCTCTCTCCCCTGCAACACACA	58°C, 50°C 93–119 bp
SLC11A1 (NRAMP1)	Promoter, microsatellite (GT)n	Fluorescence PCR	F: FAM-TTCTGTGCCTCCCAAGTTAGC R: ACTCGCATTAGGCCAACGAG	52°C 180–220 bp
	Exon 2, 9 bp del	Fluorescence PCR	F: GTGACAAGGGTCCCCAA R: TET-CGGTTTTGTGTCTGGGAT	60°C 143, 132 bp
	3′ UTR, TGTG ins/del	Fluorescence PCR	F: GCATCTCCCCAATTCATGGT R: TET-AACTGTCCCACTCTATCCTG	60°C 240, 244 bp
	3′ UTR CAAA ins/del	Fluorescence PCR	F: TET-CCTAGCGCAGCCATGTGATTACCC R: AGCCTGTGTCCCGCCCAAGTCCT	58°C 234, 238 bp
TLR2	Intron 2, microsatellite	Fluorescence PCR	F: TATCCCCATTCATTCGTTCCATT R: FAM-ACCCCCAAGACCCACACC	58°C 213–243 bp

* LDR = ligation detection reaction; PCR = polymerase chain reaction; CARD15 = caspase recruitment family, member 15; RFLP = restriction fragment length polymorphism; bp = basepairs; CCL3 = chemokine (C-C motif) ligand 3: IFNG = interferon-γ; ARMS = amplification refractory mutation system; LTA = lymphotoxin α; UTR = untranslated region; TNF-β = tumor necrosis factor β; SLC11A1 = solute carrier family 11, member 1; del = deletion; ins = insertion; TLR2 = toll-like receptor 2; fs = frameshift.

TABLE 2

Oligonucleotides and conditions required for ligation detection reaction (LDR) genotyping*

Gene	SNP location Base change Amino acid change (SNP name)†	PCR primer sequences	PCR temperature [$MgCl_2$] and product length	LDR probe sequences‡	LDR product length§ and reaction temperature
CRI	Exon 19	F: TGCGTTGGATCTTTCCCATG	63°C	P1: FAM-aaattaaaatTGGTGCATGTGATCACAGACATCCAG	81 bp
(CD35)	3093G→T	R: CCTGGTTTCCAGCAAGGATACA	2 mM	P2: HEX-taaattaaaaATGGTGCATGTGATCACAGACATCCAT	82 bp
	Q1022H		892 bp	P3: GTTGGATCCAGAATCAACTATTCTTGTACTACAGGaaataaaaaa	70°C
	Exon 28	F: TAAAAAATAAGCTGTTTTACCATACTC	61°C	P1: FAM-aataCAGCCCTCCCCCTCGGTGTATTTCTACTAATA	71 bp
	4795A→G	R: CCCTCACACCCAGCAAAGTC	1 mM	P2: HEX-ataaAGCCCTCCCCCTCGGTGTATTTCTACTAATG	70 bp
	K1590E (*McC*^{a}→*McC*^{b})		476 bp	P3: AATGCACAGCTCCAGAAGTTGAAAATGCAATatat	68°C
	Exon 29	F: TAAAAAATAAGCTGTTTTACCATACTC	61°C	P1: HEX-GAAACAGGAGTTTCTTTTCCCTCACTGAGATA	
	4870A→G	R: CCCTCACACCCAGCAAAGTC	1 mM	P2: FAM-AAACAGGAGTTTCTTTTCCCTCACTGAGATCG	
	I1615V		476 bp	P3: TCAGATTTAGATGTCAGCCCGGGTTTGTC	68°C
	Exon 29	F: TAAAAAATAAGCTGTTTTACCATACTC	61°C	P1: HEX-GAGGGAAAAGAAACTCCTGTTTCCTGGTACTCC	65 bp
	4828G→A	R: CCCTCACACCCAGCAAAGTC	1 mM	P2: FAM-GTGAGGGAAAAGAAACTCCTGTTTCCTGGTACTCT	67 bp
	R1601G (*SL1*→*S12*)		476 bp	P3: AATTGCATTTTCAACTTCTGGAGCTGTGCATT	68°C
	Exon 33	F: AAGCGCACAGTCACAGGTCAC	63°C	P1: FAM-taaaaaatataataCATCCGCTGCACAAGTGACCC	72 bp
	5507C→G	R: GGAGGTAGTTCTGTCTCTGAC	2 mM	P2: HEX-ataaaaaatataataCATCCGCTGCACAAGTGACCG	73 bp
	P1827R		443 bp	P3: TCATGGGAATGGGGTTTGGAGCtaaaaaaaaaaaaaa	64°C
ICAM1	Exon 2	F: TGTCCCCCTCAAAAGTCATCCTG	53°C	P1: FAM-CAGCACCTCCTGTGACCAGCCCAT	55 bp
	179A→T	R: TTCCCAGGCAGGAGCAACTCCTT	1 mM	P2: TET-aAGCACCTCCTGTGACCAGCCCAA	55 bp
	K29M (ICAM-1^{ref} →ICAM-1^{Kilifi})		130 bp	P3: GTTGTTGGGCATAGAGACCCCGTTGCCattt	64–68°C
IL10	Promoter	F: CTGGCTCCCCTTACCTTCTACACA	60°C	P1: FAM-CCAAGACAACACTACTAAGGCTTCTTTGGGAA	68 bp
	–1082A→G	R: TGGGCTAAATATCCTCAAAGTTCC	1.5 mM	P2: TET-CCAAGACAACACTACTAAGGCTTCTTTGGGAG	68 bp
			658 bp	P3: GGGGAAGTAGGGATAGGTAAGAGGAAAGTAAGGGAC	64–68°C
	Promoter	F: CTGGCTCCCCTTACCTTCTACACA	60°C	P1: FAM-GCACTGGTGTACCCTTGTACAGGTGATGTAAT	71 bp
	–819T→C	R: TGGGCTAAATATCCTCAAAGTTCC	1.5 mM	P2: TET-GCACTGGTGTACCCTTGTACAGGTGATGTAAC	71 bp
			658 bp	P3: ATCTCTGTGCCTCAGTTTGCTCACTATAAAATAGAGACG	64–68°C
	Promoter	F: CTGGCTCCCCTTACCTTCTACACA	60°C	P1: FAM-AACACATCCTGTGACCCCGCCTGTA	60 bp
	–592A→C	R: TGGGCTAAATATCCTCAAAGTTCC	1.5 mM	P2: TET-ttCACATCCTGTGACCCCGCCTGTC	60 bp
			658 bp	P3: CTGTAGGAAGCCAGTCTCTGGAAAGTAAAATGGAA	64–68°C
MBL2	Exon 1	F: GCACCCAGATTGTAGGACAGAG	58°C	P1: FAM-ttCGGCTTCCCAGGCAAAGATGGGC	52 bp
(MBP, MBL)	223C→T	R: CAGGCAGTTTCCTCTGGAAGG	2 mM	P2: TET-AACGGCTTCCCAGGCAAAGATGGGT	52 bp
	R52C (D variant)		339 bp	P3: GTGATGGCACCAAGGGAGAAAAGGGGG	73°C
	Exon 1	F: GCACCCAGATTGTAGGACAGAG	58°C	P1: FAM-aaaaaaaaCCAGGCAAAGATGGGCGTGATGG	62 bp
	230G→A	R: CAGGCAGTTTCCTCTGGAAGG	2 mM	P2: TET-aaaaaaaCCCAGGCAAAGATGGGCGTGATGA	62 bp
	G54D (B variant)		339 bp	P3: CACCAAGGGAGAAAAGGGGGAACCAGGaatt	64°C
	Exon 1	F: GCACCCAGATTGTAGGACAGAG	58°C	P1: FAM-ttatttaATGGGCGTGATGGCACCAAGGG	58 bp
	239G→A	R: CAGGCAGTTTCCTCTGGAAGG	2 mM	P2: FAM-tttGATGGGCGTGATGGCACCAAGGA	55 bp
	G57E (C variant)		339 bp	P3: AGAAAAGGGGGAACCAGGCCAAGGGaaat	64°C
TLR4	Exon 4	F: CTGGCTGGTTTAGAAGTCC	55°C	P1: FAM-AATTCCGATTAGCATACTTAGACTACTACCTCGATGA	83 bp
	896A→G	R: CCATCCGAAATTATAAGAAAAGT	2.5 mM	P2: HEX-TTCCGATTAGCATACTTAGACTACTACCTCGATGG	81 bp
	D299G		440 bp	P3: TATTATTGACTTATTTAATTGTTTGACAAATGTTTCTTCATTTTCC	54°C
TNF	Promoter	F: GTGGGGAGAACAAAAGGATAAG	58°C	P1: FAM-attaCAAAGGAGAAGCTGAGAAGATGAAGGAAAAGT	71 bp
	–1031T→C	R: GATGTGGCGTCTGAGGGTTGTTTT	1 mM	P2: TET-attaaAAAGGAGAAGCTGAGAAGATGAAGGAAAAGC	71 bp
			1,240 bp	P3: CAGGGTCTGGAGGGGCGGGGaaaaaaaataaataa	64°C

	Promoter	F: GTGGGGAGAACAAAAGGATAAG	58°C	P1: FAM-taaaaataGAAAGTCGAGTATGGGGACCCCCC		69 bp
	–863C→A	R: GATGTGGCGTCTGAGGGTTGTTTT	1 mM	P2: TET-taaaaataGAAAGTCGAGTATGGGGACCCCCA		69 bp
			1,240 bp	P3: CTTAACGAAGACAGGGCCATGTAGAGGGMaaataaat		64°C
	Promoter	F: GTGGGGAGAACAAAAGGATAAG	58°C	P1: FAM-aataatAAGTCGAGTATGGGGACCCCCMCTTAAC		65 bp
	–857C→A	R: GATGTGGCGTCTGAGGGTTGTTTT	1 mM	P2: TET-aataatAAGTCGAGTATGGGGACCCCCMCTTAAT		65 bp
			1,240 bp	P3: GAAGACAGGGCCATGTAGAGGGCCCataaat		64°C
	Promoter	F: GTGGGGAGAACAAAAGGATAAG	58°C	P1: FAM-GTTCTATCTTTTTCCTGCATCCTGTCTGGAAA		63 bp
	–376G→A	R: GATGTGGCGTCTGAGGGTTGTTTT	1 mM	P2: TET-GTTCTATCTTTTTCCTGCATCCTGTCTGGAAG		63 bp
			1,240 bp	P3: TTAGAAGGAAACAGACCACAGACCTGGTCCC		64°C
	Promoter	F: GTGGGGAGAACAAAAGGATAAG	58°C	P1: FAM-aaAGGCAATAGGTTTTGAGGGGCATGG		54 bp
	–308G→A	R: GATGTGGCGTCTGAGGGTTGTTTT	1 mM	P2: TET-aGAGGCAATAGGTTTTGAGGGGCATGA		53 bp
	(TNF*1→TNF*2)		1,240 bp	P3: GGACGGGGTTCAGCCTCCAGGGaaaa		64°C
	Promoter	F: GTGGGGAGAACAAAAGGATAAG	58°C	P1: FAM-aaCCAGAAGACCCCCCTCGGAATCG		51 bp
	–238G→A	R: GATGTGGCGTCTGAGGGTTGTTTT	1 mM	P2: TET-aCCCAGAAGACCCCCCTCGGAATCA		51 bp
			1,240 bp	P3: GAGCAGGGAGGATGGGGAGTGTGAGG		64°C
	Exon 1	F: GTGGGGAGAACAAAAGGATAAG	58°C	P1: FAM-taataatGACCAGCTAAGAGGGAGAGAAGCAACTACAGAC		75 bp
	C insertion at 70	R: GATGTGGCGTCTGAGGGTTGTTTT	1 mM	P2: TET-aatataatCCAGCTAAGAGGGAGAGAAGCAACTACAGACC		75bp
			1,240 bp	P3: CCCCCCTGAAAACAACCCTCAGACGaatataaaaa		64°C
VDR	Intron 8	F: CTGGGGAGCGGGGAGTATGAAGGA	58°C	P1: FAM-GGGTGGTGGGATTGAGCAGTGAGGT		67 bp
	G→T	R: GGGTGGCGGCAGCGGATGTA	1 mM	P2: HEX-aGGTGGTGGGATTGAGCAGTGAGGG		67 bp
	(*Apa* I; a→A)		1,100 bp	P3: GCCCAGCTGAGAGCTCCTGTGCCTTaaaaaaataaaaaaaaa		72°C
	Intron 8	F: CTGGGGAGCGGGGAGTATGAAGGA	58°C	P1: FAM-CAAGAGCAGAGCCTGAGTATTGGGAATGT		65 bp
	C→T	R: GGGTGGCGGCAGCGGATGTA	1 mM	P2: HEX-aAAGAGCAGAGCCTGAGTATTGGGAATGC		65 bp
	(*Bsm* I; b→B)		1,100 bp	P3: GCAGGCCTGTCTGTGGCCCCAGattaaaaaaaata		72°C
	Exon 9	F: CTGGGGAGCGGGGAGTATGAAGGA	58°C	P1: FAM-TGCAGGACGCCGCGCTGATT		70 bp
	T→C	R: GGGTGGCGGCAGCGGATGTA	1 mM	P2: HEX-aGCAGGACGCCGCGCTGATC		70 bp
	352 silent		1,100 bp	P3: GAGGCCATCCAGGACCGCCTGTCattatataaaatataaatataaaaaaa		72°C
	(*Taq* I; T→t)					

* CR = complement receptor; ICAM1 = intercellular adhesion molecule 1; IL10 = interleukin-10; MBL = mannose-binding lectin; VDR = vitamin D receptor; SNP = single nucleotide polymorphism. For definition of other abbreviations, see Table 1.

† SNP name used by previous investigators.

‡ Non-specific polynucleotides, added to LDR probes to obtain desired ligation product lengths, are shown in lower case. P1 = allelic probe 1, P2 = allelic probe 2, P3 = common probe.

§ Apparent LDR product lengths obtained using an ABI 3700 with Rox 500 size standards may be less than the absolute length of the LDR product. This discrepancy is not observed using an ABI 373 with GS-350 size standards.

scribed later in this report (Segal S and others, unpublished data).[20,21] The PCR primers, conditions, and restriction enzymes used are shown in Table 1.

Direct sequence analysis. Direct sequence analysis was used to determine whether the TLR2 R677W, R753Q, and CARD15 R790Q variants were present in the Malawi population[13,22] (National Center for Biotechnology Information SNP database, Bethesda, MD). The PCR primers and thermocycles were TLR2 exon 3 586-basepair (bp) segment: 5′-gcgtggccagcaggttcag-3′, 5′-gggccactccaggtaggtcttg-3′ (thermocycles: one cycle at 94°C for four minutes; 36 cycles at 94°C for 30 seconds, 58°C for 40 seconds, and 72°C for 60 seconds; and one cycle at 72°C for two minutes) and CARD15 exon 4 509-bp segment: 5′-gccgagccgcacaacctt-3′, 5′-gtgctcccccatacctgaaca-3′ (thermocycles: one cycle at 94°C for five minutes; 35 cycles at 60°C for 30 seconds, and 72°C for 45 seconds; and one cycle at 72°C for four minutes). The PCR (2 × 25 μL) and sequencing (20 μL) reaction were performed using standard conditions. Sequence analysis was performed using an ABI 3700 fluorescent DNA sequencer. Each segment was sequenced in both directions.

Genotyping using the post-PCR multiplexed ligation detection reaction (LDR). The LDR was used to genotype the remaining 23 SNPs located in seven genes.[23] Briefly, fragments containing the SNPs of interest were PCR-amplified using appropriate primers and conditions (Table 2). Amplifications were performed in a total volume of 15 μL and contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1–2.5 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, approximately 50 ng of DNA, and 0.5 units of Amplitaq Gold polymerase (Perkin-Elmer, Boston, MA). To prevent polymerase extension during the subsequent LDR procedure, residual polymerase activity was removed by incubation with 1:10 volume of 1 mg/mL of proteinase K in 50 mM EDTA at 37°C for 30 minutes and 55°C for 10 minutes. The proteinase K was inactivated by incubation at 99°C for 10 minutes.

A set of three probes consisting of two fluorescently labeled allelic and one common probe was designed for each of the SNPs investigated using the LDR (Table 2). The common probes (100 pmol of each) were 5′ phosphorylated in a volume of 100 μL using 10 units of T4 polynucleotide kinase and 1 mM ATP in 70 mM Tris-HCl buffer, pH 7.6, containing 10 mM $MgCl_2$ and 5 mM dithiothreitol. After incubation at 37°C for 45 minutes, the T4 kinase was inactivated by the addition of 20 μg of proteinase K in 100 μL of TE buffer (10 mM Tris-HCl, 5 mM EDTA, pH 8.0) and incubating at 37°C for 30 minutes. The proteinase K was inactivated by incubation at 99°C for 10 minutes.

The multiplexed LDR was performed in 15-μL volumes of 20 mM Tris-HCl buffer, pH 7.6, containing 25 mM KCl, 10 mM $MgCl_2$, 1 mM NAD^+, 10 mM dithiothreitol, 0.1% Triton X-100, 10 nM (200 fmol) of each LDR probe, 2 μL of PCR product, and 2 units of *Taq* DNA ligase. The reactions were prepared on ice and then initiated by placing on PCR blocks pre-heated to 95°C. An initial one-minute denaturation at 95°C was followed by 15 thermal cycles of denaturation at 95°C for 15 seconds, and annealing and ligation at the appropriate temperature (Table 2) for four minutes. The activity of the ligase was stopped after 15 cycles by cooling to 4°C and adding 2 μL of 100 mM EDTA.

Electrophoresis and detection of multiplexed and/or pooled LDR or fluorescent PCR products was performed on an ABI 373 or ABI 3700 fluorescent DNA sequencer. For the ABI 3700, 1 μL of LDR product was added to 10 μL of Hi-Di formamide and 0.01 μL of 8 nM ABI Genescan-500 Rox size standards (Applied Biosystems). For the ABI373, 2 μL of LDR product was added to 1.5 μL of 5:1 deionized formamide, 25 mM EDTA with blue dextran (1 mg/mL), and 0.5 μL of fluorescently labeled size standards (GeneScan-350 tetramethylrhodamine [TAMRA]). After denaturation, the product was subjected to electrophoresis on 6% polyacrylamide gels. Product sizes were calculated relative to the standard with Genescan 3.5 (Perkin Elmer Applied Biosystems, Warrington, United Kingdom) using the second order least squares method. Automated allele calling of LDR products was performed using Genotyper 2.5 (Perkin Elmer Applied Biosystems).

Statistical analysis. For each polymorphism, the percentage of individuals with a particular genotype was calculated separately for cases and controls. Conditional logistic regression was used to quantify the association between disease and genotype, with and without controlling for ethnic group, and with and without restriction to PB cases. The percentage of cases with a particular genotype was also calculated separately for PB and MB cases.

RESULTS

Recruitment. A total of 334 cases and 702 controls were selected from the database and sought in the field. Of the 334 cases, 22 had left the district and 34 had died by the time they were sought for genetics testing, 7 were alive but neither a blood nor a buccal swab specimen were provided, and for one case data were not available for a matched control. This gave a total of 270 cases that could be included in analyses, 26 of which were MB. Of the 702 controls that were sought, 74 had left the district and 90 had died by the time they were sought for genetics testing, 26 were alive but neither a blood nor a buccal swab specimen were provided, and for 60 data on the matched case were not available. Thus, 452 controls were included in these analyses. There were one or two controls for each case. Blood specimens were available for all except eight individuals, who provided buccal swabs.

Direct sequence analysis. Sequence analysis of 83 individuals (including cases and controls) indicated that the TLR2 R677W and R753Q variants were not present, or were very rare, in northern Malawi. Likewise, the CARD15 SNP R790Q was not found upon sequencing of 63 individuals. Accordingly, further genotyping of these SNPs was not performed.

Genotyping results. The PCR-RFLP analysis of more than 150 Malawians indicated that the CARD15 variants P268S, R702W, G908R, and 1007fs were not present in this population. The TNF-1031 variant was also not found. The MBL2 R52C, MBL2 G54D, TNF + 70, and −857 variants were very rare in Karonga District, occurring with an allele frequency of < 1.0%. No homozygotes of these variants were identified.

Association study results for leprosy susceptibility. The genotype frequencies and odds ratios (ORs) of the 26 variants that were sufficiently polymorphic for leprosy association analysis are shown in Table 3, with ORs controlled for ethnic group. The results shown combine PB and MB cases, but all results were unchanged when restricted to PB cases. For most polymorphisms, there was no evidence of an association with

TABLE 3

Genotype frequencies for all leprosy (paucibacillary and multibacillary)* cases and controls, and odds ratios (ORS) controlled for age, sex, area of residence, and ethnic group†

Gene	Polymorphism	Genotype	Case		Control		OR (95% confidence interval)
			%	n	%	n	
CCL3	Promoter microsatellite	331/331	41	54	46	131	
(MIP1-α)	$(TA)_n$ at –906	331/327	31	40	25	71	1.4 (0.7–2.7), $P = 0.37$
		331/333	16	21	18	52	0.9 (0.4–2.0), $P = 0.79$
		Other	12	16	11	31	1.0 (0.4–2.9), $P = 0.94$
				131		285	(110, 146)‡
CR1	Exon 19	g/g	79	131	81	151	
	3093G→T	g/t	20	33	17	31	1.1 (0.5–2.1), $P = 0.86$
	Q1022H	t/t	1	2	2	4	0.4 (0.06–2.6), $P = 0.33$
				166		186	(136, 136)
	Exon 28	a/a	65	164	60	238	
	4795A→G	a/g	32	80	34	135	0.8 (0.5–1.1), $P = 0.18$
	K1590E	g/g	3	7	6	26	0.3 (0.1–0.8), $P = 0.02$
	(McC^a→McC^b)			251		399	(245, 374)
	Exon 29	g/g	45	114	49	196	
	4828G→A	g/a	45	114	40	157	1.3 (0.9–1.9), $P = 0.12$
	R1601G	a/a	10	24	11	42	1.0 (0.5–1.9), $P = 0.99$
	(*Sl1*→*Sl2*)			252		395	(246, 371)
	Exon 29	g/g	76	192	79	311	
	4870A→G	g/a	23	57	20.5	81	1.3 (0.9–2.0), $P = 0.21$
	I1615V	a/a	1	3	0.5	2	1.7 (0.3–11.1), $P = 0.58$
				252		394	(246, 370)
	Exon 33	c/c	71	128	79	172	
	5507C→G	c/g	28	51	18	40	1.4 (0.8–2.5), $P = 0.21$
	P1827R	g/g	1	2	3	7	0.2 (0.01–1.7), $P = 0.12$
				181		219	(153, 168)
ICAM1	Exon 2	a/a	54	112	55	134	
	179A→T	a/t	32	68	31	76	1.1 (0.6–1.7), $P = 0.83$
	K29M	t/t	14	29	14	35	1.0 (0.5–2.0), $P = 0.93$
	(ICAM-1^{ref}→ICAM-1^{Kilifi})			209		245	(189, 208)
IFNG	Intron 1	a/a	67	157	66	265	
	874T→A	a/t	28	67	27	109	1.1 (0.7–1.6), $P = 0.72$
		t/t	5	12	7	28	0.8 (0.4–1.8), $P = 0.59$
				236		402	
IL10	Promoter	c/c	46	97	43	157	
	–592C→A	c/a	38	81	47	169	0.7 (0.5–1.1), $P = 0.13$
		a/a	16	34	10	36	1.7 (1.0–3.3), $P = 0.06$
				212		362	(203, 296)
	Promoter	c/c	44	95	42	147	
	–819C→T	c/t	41	88	48	170	0.8 (0.5–1.2), $P = 0.23$
		t/t	15	32	10	36	1.6 (0.9–3.0), $P = 0.11$
				215		353	(204, 293)
	Promoter	a/a	45	86	43	149	
	–1082A→G	a/g	44	84	44	154	1.0 (0.6–1.5), $P = 0.96$
		g/g	11	21	13	46	0.7 (0.4–1.4), $P = 0.30$
				191		349	(183, 262)
LTA	5′UTR microsatellite	101 absent	89	164	89	296	
(TNF-β)	$(AC/GT)_n$ at –3.5 kb§	101 1 copy	10	18	11	36	0.9 (0.5–1.7), $P = 0.80$
		101 2 copies	1	2	0.3	1	
				184		333	
		103 absent	52	96	59	196	
		103 1 copy	45	83	36	121	1.2 (0.8–1.8), $P = 0.42$
		103 2 copies	3	5	5	16	
				184		333	
		105 absent	49	90	58	192	
		105 1 copy	42	78	37	124	1.6 (1.1–2.4), $P = 0.03$
		105 2 copies	9	16	5	17	
				184		333	
		111 absent	95	174	90	301	
		111 1 copy	5	10	10	32	0.4 (0.2–1.1), $P = 0.07$¶
		111 2 copies	0	0	0	0	
				184		333	(163, 228)
MBL2	Exon 1	g/g	63	146	65	169	
(MBL)	239G→A	g/a	32	73	30	78	1.1 (0.7–1.7), $P = 0.63$
	G57E (C variant)	a/a	5	12	5	14	0.9 (0.4–2.1), $P = 0.73$
				231		261	(224, 238)

TABLE 3
Continued

Gene	Polymorphism	Genotype	Case %	Case n	Control %	Control n	OR (95% confidence interval)
SLC11A1	Promoter microsatellite	199/199	63	158	56	237	
(NRAMP1)	(GT)n	199/201	30	75	36	153	0.7 (0.5–1.0), $P = 0.06$
	(199 = allele 3, 201 = allele 2)	201/201	4	11	6	25	0.6 (0.3–1.4), $p = 0.24$
		Other	2	5	2	8	1.4 (0.4–4.3), $P = 0.60$
				249		423	(247, 388)
	Exon 2	Ins/ins	93	228	91	258	
	9 bp del	del present	7	16	9	25	0.7 (0.3–1.4), $P = 0.31$
				244		283	(236, 261)
	3′UTR	Ins/ins	47	122	50	203	
	TGTG ins/del	Ins/del	43	112	41	164	1.1 (0.8–1.5), $P = 0.64$
		del/del	9	24	9	35	1.2 (0.7–2.1), $P = 0.58$
				258		402	(248, 391)
	3′UTR	del/del	41	106	45	192	
	CAAA ins/del	Ins/del	45	117	44	191	1.1 (0.8–1.6), $P = 0.48$
		Ins/ins	14	35	11	46	1.5 (0.9–2.6), $P = 0.14$
				258		429	(257, 410)
TLR2	Intron 2 microsatellite#	216 absent	90	188	88	332	
		216 1 copy	10	22	12	45	0.7 (0.4–1.3), $P = 0.25$
		216 2 copies	0	0	0.5	2	
				210		379	
		222 absent	78	164	79	299	
		222 1 copy	21	44	19	74	0.9 (0.6–1.5), $P = 0.73$
		222 2 copies	1	2	2	6	
				210		379	
		224 absent	37	78	40	151	
		224 1 copy	52	108	50	188	1.0 (0.7–1.4), $P = 0.91$
		224 2 copies	11	24	10	40	
				210		379	
		226 absent	76	160	74	279	
		226 1 copy	21	44	24	92	0.9 (0.6–1.4), $P = 0.68$
		226 2 copies	3	6	2	8	
				210		379	(194, 286)
TLR4	Exon 4	a/a	95	223	91	262	
	896A→G	g/a	5	12	9	25	0.5 (0.2–1.1), $P = 0.08$
	D299G	g/g	0	0	0.4	1	
				235		288	(243, 383)
TNF	Promoter	g/g	85	206	84	237	
	–238G→A	g/a	15	36	15	43	0.9 (0.5–1.5), $P = 0.71$
		a/a	0.4	1	1	3	1.4 (0.1–16.6), $P = 0.80$
				243		283	(235, 262)
	Promoter	g/g	80	173	78	201	
	–308G→A	g/a	19	42	20	51	1.5 (0.9–2.5), $P = 0.15$
	(TNF*1→TNF*2)	a/a	1	1	2	6	0.2 (0.02–1.8), $P = 0.15$
				216		258	(195, 223)
	Promoter	g/g	88	211	86	242	
	–376G→A	g/a	11	27	13	38	0.8 (0.4–1.4), $P = 0.37$
		a/a	0.4	1	1	2	1.3 (0.1–15.8), $P = 0.83$
				239		282	(231, 257)
	Promoter	c/c	75	177	75	212	
	–863C→A	a/c	22	51	24	68	0.9 (0.6–1.5), $P = 0.77$
		a/a	3	8	1	3	4.0 (0.8–19.7), $P = 0.09$
				236		283	(228, 254)
VDR	Intron 8	t/t	50	85	45	147	
	G→T	t/g	43	73	48	158	0.7 (0.5–1.2), $P = 0.21$
	(*Apa* I; a→A)	g/g	7	11	7	23	1.5 (0.6–4.0), $P = 0.43$
				169		328	(159, 235)
	Intron 8	c/c	62	104	60	197	
	C→T	c/t	28	47	35	115	0.8 (0.5–1.3), $P = 0.35$
	(*Bsm* I; b→B)	t/t	10	17	5	18	2.1 (0.9–5.0), $P = 0.08$
				168		330	(158, 235)
	Exon 9	t/t	53	131	57	228	
	T→C (codon 352)	t/c	41	101	40	158	1.1 (0.8–1.7), $P = 0.54$
	(*Taq* I; T→t)	c/c	6	15	3	12	4.3 (1.6–11.4), $P = 0.004$
				247		398	(240, 375)

* The results did not change when analysis was restricted to paucibacillary cases and their matched controls.
† For definitions of abbreviations, see Tables 1 and 2.
‡ The number of cases and controls used in the logistic regression.
§ ORs are for one copy of the allele, and are with respect to baseline of pairs of alleles not including any of 101, 103, 105, and 111.
¶ $P = 0.03$, test for heterogeneity among LTA alleles.
# ORs are for one copy of the allele, and are with respect to baseline of pairs of alleles not including any of 216, 222, 224, and 226.

leprosy susceptibility; however, for TNF −376 and −238, the 95% confidence intervals (CIs) were too wide to eliminate this. The polymorphisms ICAM1 K29M, MBL2 G57E, SLC11A1 exon 2 9-bp deletion, TGTG insertion/deletion, CAAA insertion/deletion, CR1 R1601G, IFNG +874, CCL3 microsatellite, TLR2 intron 2 microsatellite, and IL10 -1082 were not found to be associated with leprosy susceptibility. Borderline evidence of association with leprosy susceptibility was found for six polymorphisms: TNF −863, SLC11A1 promoter microsatellite, IL10 −592, TLR4 D299G, VDR *Bsm* I, and the LTA microsatellite 111-bp allele. Two SNPs were found to be associated with leprosy susceptibility with a 5% level of statistical significance without correcting for multiple comparisons: homozygotes (tt) for a silent T→C change in codon 352 of the VDR gene are susceptible (OR = 4.3, 95% CI = 1.6–11.4, $P = 0.004$), while homozygotes for the CR1 variant K1590E are protected (OR = 0.3, 95% CI = 0.1–0.8, $P = 0.02$). Presence of the LTA microsatellite 105-bp allele, but not the overall distribution of alleles at this microsatellite locus, was also associated with leprosy susceptibility (OR = 1.6, 95% CI = 1.1–2.4, $P = 0.03$).

Association study results for leprosy type. Comparison of genotype frequency between MB and PB cases usually indicated no differences. However, there was suggestive evidence that presence of the LTA 101-bp microsatellite allele is associated with MB leprosy ($\chi^2 = 11.4$, $P = 0.003$) and borderline evidence that the TLR2 224-bp microsatellite allele ($\chi^2 = 6.3$, $P = 0.042$) genotype frequencies may differ between leprosy types. However, since only 26 MB cases were included in this study these results could be due to sampling error.

DISCUSSION

Of the 38 polymorphisms investigated, 12 were insufficiently polymorphic for analysis and 2 showed evidence of association with leprosy susceptibility in this population. These two are discussed first.

The nuclear hormone receptor VDR mediates the many immunomodulatory effects of the active form of vitamin D ($1\alpha25(OH)_2D3$), which include suppression of cytokine synthesis, immunoglobulin production, and lymphocyte proliferation. Epidemiologic evidence suggests that vitamin D deficiency may be associated with susceptibility to tuberculosis and vitamin D impairs growth of *M. tuberculosis* in human macrophage and monocytic cell lines, while vitamin D-containing medications have been useful in treating some leprosy cases.[10,24] Homozygosity for a *Taq* I polymorphism (tt) in the ligand binding domain of VDR was associated with PB leprosy in Bengali Indians and resistance to tuberculosis in The Gambia.[10,25] In the Bengali leprosy study the TT genotype was associated with MB leprosy and the Tt genotype was associated with leprosy susceptibility *per se*. In Karonga, where the leprosy cases are predominantly (90%) PB, the tt genotype was associated with susceptibility to leprosy *per se*. However, the apparent association in Karonga should be interpreted with caution. The expected frequency of tt homozygotes based on the measured t allele frequency in controls is 5%. The observed frequency in cases is 6% and in controls 3%. Thus, much of the apparent difference relates to a deviation of the control frequency from Hardy-Weinberg equilibrium, which may be a chance finding. In Mali, no association was found for the VDR genotype with either lepromatous or tuberculoid leprosy or leprosy *per se* (Meisner S, unpublished data). It is not clear whether the silent T/t polymorphism has a direct effect on function or expression of VDR, and it seems more likely that reported disease associations, if real, relate to linkage disequilibrium with a flanking functional polymorphism.[26,27]

On phagocytic cells, CR1 mediates the adherence and phagocytosis of complement-opsonized pathogens. Since *M. leprae* and *M. tuberculosis* can both gain entry into macrophages using this mechanism, it was hypothesized that variants in CR1 may influence susceptibility to mycobacterial disease.[28,29] Five polymorphisms that encode amino acid changes in the extracellular domains of the protein and may affect either ligand binding or CR1 stability were investigated.[30,31] These included the variants K1590E and R1601G, which underlie the Knops blood group McCoy ($McC^a \rightarrow McC^b$) and Swain-Langley ($Sl1 \rightarrow Sl2$) polymorphisms, respectively. Both the McC^b and *Sl2* alleles are more prevalent in African populations than in Caucasian, Asian, or Hispanic Americans, and it has been suggested that they may confer a selective advantage against infectious disease.[32] These alleles were less common in Malawi ($McC^b = 0.23$, $Sl2 = 0.31$) than in the west African Gambian population ($McC^b = 0.39$, $Sl2 = 0.80$), but more common than in non-African populations ($McC^b \leq 0.025$, $Sl2 \leq 0.03$). Homozygosity for the McC^b allele was associated with protection against leprosy in Malawi; however, the biologic mechanism underlying this finding is unclear.

Four other genes, TNF, LTA, IL-10, and TLR2, which have been reported to be associated with leprosy susceptibility elsewhere, failed to show association of the relevant polymorphisms in Karonga.[3]

The proinflammatory cytokine TNF influences several aspects of the immune response, including macrophage activation (important for killing of mycobacteria) and granuloma formation (important for containment of mycobacterial infections), and can also cause tissue damage and apoptosis. Several promoter polymorphisms exist which may be involved in regulation of TNF expression and such variants have been associated with both autoimmune and infectious diseases.[33–35] A G→A polymorphism (TNF*2) at −308, which may be associated with increased TNF expression, was strongly linked to and associated with resistance to leprosy in Brazil.[6,9,36,37] Contrary to these findings, the same variant has been associated with MB leprosy susceptibility in Bengali Indians, indicating, perhaps, that different aspects of TNF may be important in controlling the outcome of leprosy infection in different populations, perhaps due to the presence of different *M. leprae* strains, or a modified cytokine milieu resulting from presence of varying infectious pathogens or differences in genetic backgrounds.[38] We found no evidence that the −308 variant has a role in leprosy susceptibility in Malawi. Six other TNF polymorphisms were investigated in this study, but the −1031, +70 and −857 variants were very rare and thus could not be tested for any association. There was no evidence that the −376 polymorphism, which upregulates TNF expression by introducing a binding site for the OCT-1 transcription factor, and is associated with severe malaria, was associated with leprosy susceptibility in Malawi, although the confidence limits were too wide to eliminate this possibility.[33] Consistent with a recent Brazilian leprosy study, the −238

SNP, which may produce increased TNF transcription, was not found to be associated with leprosy in Malawi.[9,39] The –863 SNP is located in an NF-κB regulatory site and results in reduced p50-p50 enhancement of expression.[40] There was borderline evidence that this variant may be associated with leprosy susceptibility in Malawi, which is consistent with the findings that higher levels of TNF production may be protective against leprosy in Brazil.

Located close to the TNF gene, LTA encodes a cytokine that is produced by lymphocytes and natural killer cells and which has pleiotropic immunomodulatory effects. A haplotype that includes an intronic LTA SNP is associated with leprosy susceptibility in Brazil.[6] In Malawi, the 105-bp allele may be weakly associated with leprosy susceptibility, while the 101-bp allele may influence leprosy type. It is possible that these associations are due to linkage disequilibrium with other polymorphisms either in another gene in the HLA region, or within LTA, or they may just be chance findings.[20] However, further investigation of possible associations, if any, between LTA and susceptibility both to leprosy *per se*, and to leprosy type is warranted.

The cytokine IL10 has pleiotropic effects in immunoregulation and inflammation, including the inhibition of TH1 cytokine secretion and T cell proliferation. Polymorphisms in the promoter influence the amount of IL10 production.[10,41,42] It was speculated that by suppressing inflammation, high IL10 production might increase mycobacterial susceptibility. Three promoter polymorphisms in IL10 were investigated in Malawi and borderline evidence was found that homozygotes for the –592 A variant may have increased susceptibility to leprosy. Recently, the -819 T variant has been reported to be weakly associated with leprosy susceptibility in Brazil.[9] These two variants exhibit linkage disequilibrium and usually occur together on a haplotype (–1082 A, –1892 T, –592 A) that is associated with increased IL10 production. In Brazil, homozygosity for the –592 A variant is not significantly associated with susceptibility, but there is a trend in this direction. Thus, it is possible that the ATA haplotype may be associated with leprosy susceptibility in both these populations but much larger studies would be required to assess this.

Mammalian toll-like receptors recognize and react to a variety of bacterial cell wall components to activate direct antimicrobial mechanisms and to facilitate transcription of genes that regulate the adaptive inflammatory immune response. TLR2, probably acting as a heterodimer with other TLRs, recognizes components from a variety of microbial pathogens including mycobacterial lipoproteins and lipoarabinomannan. *Mycobacterium leprae* can activate both TLR2-TLR1 heterodimers and TLR2 homodimers.[43] TLR4 is the receptor for lipopolysaccharide (LPS), a major cell wall component of gram-negative bacteria, but it seems that both TLR2 and TLR4 can mediate cellular activation in response to components of gram-positive *Mycobacterium tuberculosis*.[44] An Arg299Gly polymorphism in the extracellular domain results in impaired TLR4-mediated LPS induced signaling.[45] Heterozygosity is associated with hypo-responsiveness to inhaled LPS, Thus, it appears counter-intuitive that although only borderline significant, heterozygosity for the rare 299Gly allele was increased among controls.[46] It would be of interest to determine whether this genotype is associated with protection in other populations.

Three TLR2 polymorphisms were also investigated. Sequencing indicated that Arg677Trp and Arg753Gln, identified as rare variants in other populations, are extremely rare in Malawi and variation in a microsatellite located in intron 2 of the TLR2 gene was not associated with leprosy susceptibility.[13,22]

Mannose-binding lectin binds to mannose and N-acetylglucosamine groups on a variety of bacteria, resulting in complement activation and opsonophagocytosis. Any one of three exon 1 point mutations (R52C, G54D, and G57E) reduces serum MBL concentrations. Since low serum levels of MBL may be associated with recurrent infections in young children and yet low-producing alleles are maintained in all populations, as for ICAM1, it has been proposed that balancing selection might exist.[47] One hypothesis is that low MBL levels may protect against mycobacterial disease by limiting phagocytotic entry of mycobacteria into host cells.[48] Consistent with studies of other African populations, the R52C and G54D polymorphisms were found only rarely in Malawi. The G57E (C) variant had an allele frequency of 20% and was not associated with leprosy susceptibility.

Interferon-γ is an essential and central regulator of the human response to infection. The intronic +874 T/A SNP lies in a putative binding site for the transcription factor NF-κB. The T allele, which may be associated with high IFN-γ production has recently been found to be associated with resistance to tuberculosis in Sicilians and South Africans.[10,19,49,50] This SNP has not previously been investigated for an association with leprosy susceptibility and was not found to be associated with leprosy in Malawi. This could be because the lower allele frequency in Malawi (20% compared with 50%) reduces the power to detect such an association because the tuberculosis association is due to another polymorphism that is in linkage disequilibrium with the +874 SNP in Sicily but not in Malawi, or because this allele does not affect leprosy susceptibility in this population.

The SLC11A1 gene encodes a protein that may have a direct effect on the survival of mycobacteria within macrophage phagosomes, but may also have pleiotropic effects that include regulation of the TH1:TH2 balance of the adaptive immune response to infection. Since the mouse homolog Slc11a1 protects against some mycobacterial infections and SLC11A1 variants have been associated with human tuberculosis susceptibility, SLC11A1 has long been a candidate for leprosy susceptibility. However, although a haplotype segregation study suggested this gene may influence leprosy susceptibility in Vietnam,[51] other studies have found no evidence for association and/or linkage with susceptibility.[25,51–54] However, variants of this gene have been associated with leprosy type in Mali and linked with the size of the Mitsuda response in southeast Asians.[53,55] For three of the four SLC11A1 polymorphisms examined in the Malawi population, there was no evidence of an association with leprosy susceptibility. The fourth, a promoter microsatellite, showed borderline evidence that the putative lower-expressing allele 201 (allele 2) may be protective against leprosy in this population. This contrasts with the reported association of this allele with tuberculosis susceptibility in The Gambia.[10,56]

The aim of this study was to better understand the genetic factors that underlie host immune responses to leprosy. We find evidence that variants in the VDR and CR1 genes may influence leprosy susceptibility in the Karonga District of northern Malawi, and it is possible that variants of some of

the others genes studied may also play a minor role. The sibling risk ratio for leprosy in this population appears to be of the order of two, and thus the genes and modest associations studied here appear insufficient to account for the overall genetic component identified.[57] Therefore, it seems likely that there are several other host genes that contribute to a complex network of gene products that may synergize or counteract each other to influence leprosy susceptibility. Interaction of strain, environment, nutrition, presence and nature of concurrent background infections, varying linkage disequilibrium, and allele frequencies may limit the usefulness of extrapolating these findings to other populations. Indeed, a major conclusion from this large-scale study is that many of the associations suggested by studies in other populations are not found in this Malawian population. Our data urge caution in the interpretation of infectious disease genetic associations from single-population analyses and support the reassessment of positive findings in replication studies.

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