Genetic variants of the MRC1 *gene and the* IFNG *gene are associated with leprosy in Han Chinese from Southwest China*

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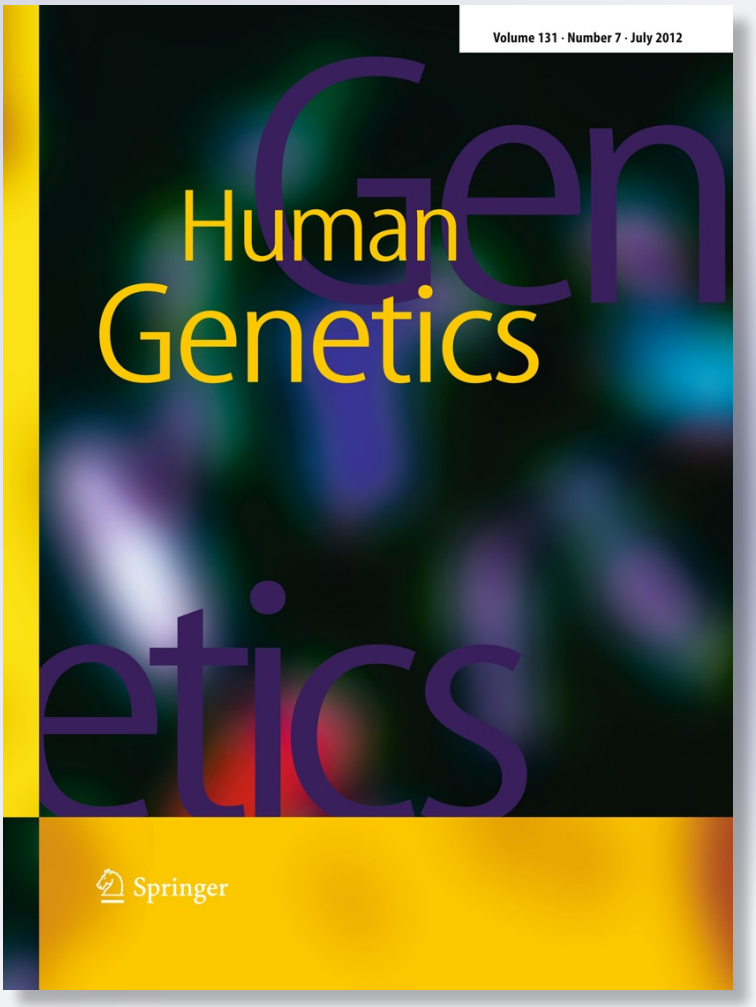

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ORIGINAL INVESTIGATION

Genetic variants of the *MRC1* gene and the *IFNG* gene are associated with leprosy in Han Chinese from Southwest China

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Abstract Leprosy is an ancient infectious disease, with over 200,000 affected people (mainly in Asia and Africa) being registered annually. Genetic factors may confer susceptibility to this disease. In the present study, we genotyped 12 genetic variants of the *MRC1* gene and the *IFNG* gene in 527 Han Chinese with leprosy and 583 healthy individuals from Yunnan, China, to discern potential association of these two genes with leprosy. In particular, we aimed to validate the recently reported association of *MRC1* variant rs1926736 (p.G396S) and *IFNG* variant rs2430561 (+874 T > A) with leprosy, which were initially observed in Vietnamese and Brazilian populations, respectively. Our results failed to confirm the reported association between variants rs1926736 and rs2430561 and leprosy in Han Chinese. However, we found that variants rs692527 ($P = 0.022$) and rs34856358 ($P = 0.022$) of the *MRC1* gene were associated with paucibacillary leprosy, and rs3138557 of the *IFNG* gene was significantly associated with multibacillary leprosy. The exact role of the *MRC1* gene and the *IFNG* gene in leprosy awaits future study.

D. Wang and J.-Q. Feng contributed equally to this work.

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Introduction

Leprosy is a chronic infectious and neurological disease that was caused by the infection of *Mycobacterium leprae* (Britton and Lockwood 2004). According to the latest information released by the World Health Organization (WHO 2010), the newly registered leprosy patients reached 211,903 at the beginning of 2010 and many regions in the world have not achieved the leprosy elimination goal. China had achieved the goal of leprosy elimination in 1981 at country level, but there are many new features concerning the epidemiological trends of leprosy in recent years in those regions that had eliminated leprosy (Li et al. 2011). Evidently, leprosy remains and will continue to be a public health problem (WHO 2010).

Because the causative agent, *M. leprae*, could not be cultured in vitro and had an eroded genome (Cole et al. 2001; Misch et al. 2010; Monot et al. 2009), the exact mechanism of leprosy has not been completely elucidated in spite of decades of research. Host genetic factors contributed to susceptibility to leprosy (Alcaïs et al. 2005;

Alter et al. 2011; Misch et al. 2010). Many genetic association studies have identified a variety of chromosomal regions, genes, and single nucleotide polymorphisms (SNPs) that affected the susceptibility to leprosy in different human populations (Alcaïs et al. 2005; Alter et al. 2011; Cardoso et al. 2011; Mira et al. 2003; Misch et al. 2010; Siddiqui et al. 2001; Tosh et al. 2002; Zhang et al. 2009, 2011). However, due to regional difference and potential population stratification, many of the reported susceptible loci and SNPs were not always validated by independent studies or in different populations. This is particularly common for case–control study for certain candidate gene(s) and also poses a daunting challenge for validating the results from the recently available genome-wide association study (GWAS).

In two recent studies, genetic polymorphisms in the *MRC1* gene (mannose receptor, C-type 1; rs1926736, c.1186A > G, p.G396S) and the *IFNG* gene (interferon-gamma; rs2430561, +874 T > A) were reported to be associated with leprosy in Vietnamese and Brazilian populations, respectively (Alter et al. 2010; Cardoso et al. 2010). These positive observations are consistent with the notion that these two genes play an active role in innate immune response which is actively involved in the pathogenesis of leprosy (Modlin 2010; Montoya and Modlin 2010). In this study, we genotyped 9 SNPs of the *MRC1* gene and 2 SNPs of the *IFNG* gene (including the two reported SNPs rs1926736 and rs2430561) in 527 Han Chinese with leprosy and 583 healthy subjects from Yunnan, China, with an intention to validate the reported association between these SNPs and leprosy. We also screened the dinucleotide-repeat polymorphism (CA repeat, rs3138557) in the first intron of the *IFNG* gene, which was said to affect the expression of IFN-γ in diseases (Awad et al. 1999; Cardoso et al. 2010; Pravica et al. 1999). We failed to validate the reported association between the SNPs rs1926736 and rs2430561 and leprosy, but discerned a positive association of SNPs rs692527 and rs34856358 of the *MRC1* gene and rs3138557 of the *IFNG* gene with leprosy subtypes.

Materials and methods

Subjects

A total of 527 leprosy patients (mean onset age 24.7 ± 12.3 years), with complete medical records, were recruited form the Yuxi Prefecture, Yunnan Province in Southwest China. Among them, 279 patients could be grouped into multibacillary leprosy (MB; including 109 lepromatous leprosy [LL], 145 borderline lepromatous leprosy [BL] and 25 borderline leprosy [BB]) and 248 into paucibacillary leprosy (PB; including 175 tuberculoid leprosy [TT] and 73 borderline tuberculoid leprosy [BT]). The diagnosis of leprosy in these patients was based on clinical and histopathological features and/or bacteriological index (if available), as had been described in our recent epidemiological study for leprosy in this region (Li et al. 2011). We enrolled 583 healthy individuals (mean age 36.0 ± 15.5 years) without any history of leprosy, HIV, and tuberculosis from the same geographic area as a control group. Informed consents conforming to the tenets of the Declaration of Helsinki were obtained from all participants prior to this study. The institutional review board of the Kunming Institute of Zoology approved this study.

SNP selection and genotyping

Genomic DNA was extracted from whole blood using the AxyPrep™ Blood Genomic DNA Miniprep Kit (Axygen, USA). Eight SNPs in the *MRC1* gene (rs2436680, rs2477637, rs2253120, rs692527, rs1926736, rs34856358, rs691461 and rs691005) and two SNPs in the *IFNG* gene (rs2430561 and rs2069718) were selected according to the SNP information in public database (NCBI dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/; HapMap, http://hapmap.ncbi.nlm.nih.gov/, phase 3, CHB) under a rational of minor allele frequency (MAF) >10%. Among them, SNPs rs2436680, rs1926736 and rs691461 of the *MRC1* gene were marked as tag SNPs in the CHB dataset of the HapMap. SNP rs34301598 of the *MRC1* gene, which is adjacent to rs1926736, was identified while we genotyped the latter SNP by sequencing.

Three genotyping methods were employed in our study. SNPs rs34301598 and rs1926736 of the *MRC1* gene and rs2430561 in the *IFNG* gene were detected by direct sequencing (Figure S1). Briefly, primer pairs 5′-GTGGC ATTTTCAGCATTTG-3′/5′-TGATGTGCCTACTCACT GTCC-3′ (for rs34301598 and rs1926736 of the *MRC1* gene) and 5′-CATCTACTGTGCCTTCCTGTAGGGT-3′/ 5′-CCGGAACTTCGTTGCTCACTGGG-3′ (for rs2430561 in the *IFNG* gene) were used for PCR amplification and sequencing. Purified PCR products were sequenced using the BigDye® v3.1 dye terminator and were analyzed on ABI PRISM™ 3730xl DNA analyzer (Applied Biosystems). We followed the same method described by Khani-Hanjani et al. (2000) to genotype the dinucleotide repeat CA in the non-coding region of the *IFNG* gene. In brief, primer pair 5′-6FAM-AGACATTCACAATTGATTTTATTCTTAC-3′ (with a fluorescent label)/5′-CCTTCCTGTAGGGTAT TATTATACG-3′ was used to amplify a short fragment (~130 bp) covering the first intron of the *IFNG* gene. About 10 μL of cocktail, which contains PCR product, Hi-Di™ Formamide and GeneScan™-500 LIZ® Size Standard, was

denatured at 95°C for 3 min and was loaded on ABI PRISM™ 3730xl DNA analyzer.

Eight SNPs were detected using multiplex PCR and the SNaPshot assay (Figure S1 and Table S1). All PCR reactions were carried out in a volume of 8 μL reaction solution containing 4–20 ng template DNA, 0.4 mM dNTPs, 0.2–0.5 μM of each primer (Table S1), 2.0 mM $MgCl_2$ and 1.0 U of AmpliTaq Gold polymerase (Applied Biosystems). The thermal amplification program consisted of one denaturation cycle at 94°C for 2 min, 40 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and ended with incubation at 4°C. PCR products were cleaned up using 1.0 U of shrimp alkaline phosphatase (SAP) and 0.5 U of Exonuclease I (TaKaRa Biotechnology Co. Ltd., Dalian, China) at 37°C for 40 min, followed by an incubation at 90°C for 10 min to inactivate the enzyme. The single-base extension reaction was performed in a total volume of 10 μL reaction solution which contains 4 μL of the above-treated PCR products, 5 μL SNaPshot Multiplex Ready Reaction Mix, and 0.4–0.8 μM pooled SNP-specific oligonucleotide primers (Table S1) according to the protocol of the ABI PRISM® SNaPshot® Multiplex Kit (Applied Biosystems). The thermal cycling program for single-base extension contained 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s. Products were treated by SAP (1.0 U) at 37°C for 40 min, followed by a heat inactivation at 75°C for 20 min. We loaded 0.5 μL of products, 9 μL of Hi-Di™ formamide and 0.5 μL of GeneScan™ 120 LIZ™ size standard (Applied Biosystems) for capillary electrophoresis on ABI PRISM™ 3730xl DNA analyzer (Applied Biosystems). The GeneMarker software (Holland and Parson 2011) was used to read the genotyping result.

Statistical analyses

Deviation from the Hardy–Weinberg equilibrium (HWE) was assessed for each variant using the Chi-square test. Cases and controls were compared for difference of genotype and allele frequencies. The linkage disequilibrium (LD) structures of the 9 SNPs of the *MRC1* gene and 2 SNPs of the *IFNG* gene were constructed using Haploview software version 4.2 (Barrett et al. 2005) according to the genotyping data of the cases and controls. We also reconstructed haplotype for SNPs in the *MRC1* and *IFNG* genes using Phase software (Stephens et al. 2001). The global difference in haplotype frequencies between the cases and controls was estimated by the Chi-square test. Potential association between certain polymorphism(s) and leprosy (including subtype) was estimated using the unconditional logistic regression model, with an adjustment of sex. All analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, Illinois). Power calculations were performed using the Quanto software (Gauderman 2002).

Results

Statistical power of the test

As MAF of all SNPs (biallelic polymorphisms) analyzed in this study ranged from 15.8 to 45.7%, while variant rs3138557 in the *IFNG* gene had many CA-repeat alleles, we restricted our estimation for statistical power based on biallelic polymorphisms only. Considering an MAF of 0.158 as observed in our samples, the power to detect odds ratio (OR) value as low as 1.6 for risk allele was expected to be above 94%, whereas the power for MAF of 0.457 was expected to be above 89%.

Lack of association of rs1926736 in the *MRC1* gene and rs2430561 in the *IFNG* gene with leprosy

The genotype and allele frequencies of 11 SNPs (nine of the *MRC1* gene and two of the *IFNG* gene) in 527 leprosy patients and 583 healthy subjects were listed in Table 1. None of these SNPs showed any deviation from HWE in both case and control populations. We constructed the linkage disequilibrium map of the tested SNPs in the case and control populations (Fig. 1). Both populations showed similar LD structure for each gene.

We observed no significant difference regarding the distribution of allele and genotype of the 11 SNPs between the case and control populations, except for rs34856358 that had a marginal significant difference ($P = 0.048$) at the genotype level (Table 1). There was no association of the reported SNPs rs1926736 in the *MRC1* gene and rs2430561 in the *IFNG* gene with leprosy (Table 1). Note that the minor allele frequency (MAF) of rs1926736 (T allele) in our samples (cases, 46.4%; controls, 45.7%), which is similar to the CHD dataset (T allele, 42.7%) of the HapMap, was much higher than that of Vietnamese (T allele, 35%) and Brazilian (T allele, 32%) (Alter et al. 2010). In contrast, MAF of rs2430561 in our samples (T allele, $<17.0\%$) was much lower than that of Brazilian population (T allele, $>30.0\%$) (Cardoso et al. 2010). We speculated that the MAF discrepancy reflected regional difference and accounted for the negative results in the current study. There was no significant difference of the three tag SNPs (rs2436680, rs691461 and rs1926736) of the *MRC1* gene between the HapMap CHB dataset (Phase 3) and our case or between the HapMap CHB dataset (Phase 3) and control population at both genotype and allele levels.

SNPs rs692527 and rs34856358 of the *MRC1* gene were associated with leprosy subtypes

When the entire leprosy patients were grouped into PB and MB populations according to their clinical expression,

Table 1 Genotype and allele frequencies of 9 SNPs in the *MRC1* gene and 2 SNPs in the *IFNG* gene in 527 leprosy patients and 583 healthy controls in Yunnan, China

SNP/location	Genotype/allele	Control	Case	Case versus control		MB versus control			PB versus control		
		No. (%)	No. (%)	OR (95% CI)*	*P* value*	No. (%)	OR (95% CI)	*P* value*	No. (%)	OR (95% CI)	*P* value*
MRC1 gene											
rs2436680	CC	116 (19.9)	125 (23.7)	Reference		61 (21.9)	Reference		64 (25.8)	Reference	
Intron 1	CT	301 (51.6)	263 (49.9)	0.801 (0.591–1.085)	0.152	139 (49.8)	0.871 (0.601–1.263)	0.467	124 (50.0)	0.733 (0.505–1.064)	0.102
tag SNP	TT	166 (28.5)	139 (26.4)	0.799 (0.568–1.123)	0.197	79 (28.3)	0.935 (0.619–1.412)	0.749	60 (24.2)	0.670 (0.437–1.028)	0.153
	C allele	533 (45.7)	513 (48.7)	Reference		261 (46.8)	Reference		252 (50.8)	Reference	
	T allele	633 (54.3)	541 (51.3)	0.901 (0.762–1.066)	0.226	297 (53.2)	0.975 (0.796–1.195)	0.807	244 (49.2)	0.826 (0.668–1.021)	0.077
HWE *P* value#		0.331	0.978								
rs2477637	CC	30 (5.1)	32 (6.1)	Reference		13 (4.7)	Reference		19 (7.7)	Reference	
intron 1	CT	234 (40.1)	209 (39.7)	0.782 (0.457–1.339)	0.370	107 (38.4)	0.992 (0.495–1.987)	0.981	102 (41.1)	0.642 (0.343–1.201)	0.166
	TT	319 (54.7)	286 (54.3)	0.815 (0.481–1.381)	0.448	159 (57.0)	1.128 (0.571–2.232)	0.729	127 (51.2)	0.611 (0.330–1.132)	0.117
	C allele	294 (25.2)	273 (25.9)	Reference		133 (23.8)	Reference		140 (28.2)	Reference	
	T allele	872 (74.8)	781 (74.1)	0.977 (0.806–1.184)	0.809	425 (76.2)	1.098 (0.866–1.391)	0.440	356 (71.8)	0.870 (0.686–1.103)	0.251
HWE *P* value		0.121	0.446								
rs2253120	CC	342 (58.7)	309 (58.6)	Reference		168 (60.2)	Reference		141 (56.9)	Reference	
exon 2	CT	215 (36.9)	186 (35.3)	0.935 (0.727–1.202)	0.599	95 (34.1)	0.873 (0.643–1.186)	0.386	91 (36.7)	1.005 (0.732–1.378)	0.977
	TT	26 (4.5)	32 (6.1)	1.426 (0.827–2.459)	0.202	16 (5.7)	1.283 (0.667–2.467)	0.456	16 (6.5)	1.545 (0.800–2.985)	0.196
	C allele	899 (77.1)	804 (76.3)	Reference		431 (77.2)	Reference		373 (75.2)	Reference	
	T allele	267 (22.9)	250 (23.7)	1.044 (0.856–1.273)	0.671	127 (22.8)	0.981 (0.770–1.249)	0.877	123 (24.8)	1.105 (0.863–1.415)	0.427
HWE *P* value		0.284	0.571								
rs692527	CC	69 (11.8)	80 (15.2)	Reference		37 (13.3)	Reference		43 (17.3)	Reference	
intron 5	CT	302 (51.8)	253 (48.0)	0.702 (0.487–1.011)	0.058	137 (49.1)	0.833 (0.531–1.306)	0.426	116 (46.8)	0.598 (0.385–0.929)	0.022
	TT	212 (36.4)	194 (36.8)	0.784 (0.536–1.145)	0.208	105 (37.6)	0.931 (0.585–1.483)	0.765	89 (35.9)	0.665 (0.420–1.051)	0.080
	C allele	440 (37.7)	413 (39.2)	Reference		211 (37.8)	Reference		202 (40.7)	Reference	
	T allele	726 (62.3)	641 (60.8)	0.944 (0.794–1.121)	0.509	347 (62.2)	1.006 (0.816–1.240)	0.957	294 (59.3)	0.882 (0.710–1.094)	0.253
HWE *P* value		0.013	0.867								
rs34301598	AA	413 (70.8)	361 (68.5)	Reference		187 (67.0)	Reference		174 (70.2)	Reference	
exon 7	AG	156 (26.8)	149 (28.3)	1.075 (0.823–1.404)	0.595	83 (29.7)	1.151 (0.836–1.584)	0.388	66 (26.6)	1.002 (0.713–1.408)	0.991
	GG	14 (2.4)	17 (3.2)	1.365 (0.661–2.822)	0.400	9 (3.2)	1.420 (0.601–3.356)	0.424	8 (3.2)	1.313 (0.538–3.204)	0.550
	A allele	982 (84.2)	871 (82.6)	Reference		457 (81.9)	Reference		414 (83.5)	Reference	
	G allele	184 (15.8)	183 (17.4)	1.106 (0.883–1.386)	0.382	101 (18.1)	1.163 (0.889–1.521)	0.270	82 (16.5)	1.050 (0.788–1.398)	0.739
HWE *P* value		0.871	0.735								
rs1926736	CC	169 (29.0)	150 (28.5)	Reference		71 (25.4)	Reference		79 (31.9)	Reference	
exon 7, tag SNP	CT	295 (50.6)	265 (50.3)	1.004 (0.761–1.325)	0.976	144 (51.6)	1.168 (0.829–1.646)	0.375	121 (48.8)	0.867 (0.615–1.222)	0.414
(p.G396S)	TT	119 (20.4)	112 (21.3)	1.081 (0.768–1.521)	0.655	64 (22.9)	1.306 (0.863–1.975)	0.206	48 (19.4)	0.867 (0.563–1.334)	0.516

Table 1 continued

SNP/location	Genotype/allele	Control	Case	Case versus control		MB versus control			PB versus control		
		No. (%)	No. (%)	OR (95% CI)*	*P* value*	No. (%)	OR (95% CI)	*P* value*	No. (%)	OR (95% CI)	*P* value*
	C allele	633 (54.3)	565 (53.6)	Reference		286 (51.3)	Reference		279 (56.2)	Reference	
	T allele	533 (45.7)	489 (46.4)	1.036 (0.876–1.226)	0.679	272 (48.7)	1.140 (0.930–1.397)	0.206	217 (43.8)	0.925 (0.747–1.144)	0.470
HWE *P* value		0.638	0.802								
rs34856358	CC	208 (35.7)	182 (34.5)	Reference		99 (35.5)	Reference		83 (33.5)	Reference	
intron 7	CT	303 (52.0)	253 (48.0)	0.936 (0.721–1.216)	0.623	137 (49.1)	0.933 (0.681–1.279)	0.667	116 (46.8)	0.939 (0.672–1.313)	0.713
	TT	72 (12.3)	92 (17.5)	1.453 (1.004–2.103)	0.048	43 (15.4)	1.238 (0.790–1.941)	0.352	49 (19.8)	1.688 (1.080–2.638)	0.022
	C allele	719 (61.7)	617 (58.5)	Reference		335 (60.0)	Reference		282 (56.9)	Reference	
	T allele	447 (38.3)	437 (41.5)	1.133 (0.954–1.344)	0.154	223 (40.0)	1.061 (0.862–1.306)	0.574	214 (43.1)	1.210 (0.977–1.500)	0.081
HWE *P* value		0.017	0.801								
rs691461	CC	204 (35.0)	183 (34.7)	Reference		96 (34.4)	Reference		87 (35.1)	Reference	
intron 7	CT	301 (51.6)	249 (47.2)	0.915 (0.704–1.190)	0.508	135 (48.4)	0.939 (0.683–1.291)	0.698	114 (46.0)	0.886 (0.635–1.236)	0.476
tag SNP	TT	78 (13.4)	95 (18.0)	1.345 (0.936–1.932)	0.109	43 (17.2)	1.273 (0.823–1.969)	0.278	47 (19.0)	1.402 (0.900–2.184)	0.135
	C allele	709 (60.8)	615 (58.3)	Reference		327 (58.6)	Reference		288 (58.1)	Reference	
	T allele	457 (39.2)	439 (41.7)	1.102 (0.929–1.307)	0.267	231 (41.4)	1.082 (0.880–1.330)	0.455	208 (41.9)	1.117 (0.901–1.385)	0.314
HWE *P* value		0.045	0.522								
rs691005	CC	53 (9.1)	59 (11.2)	Reference		34 (12.2)	Reference		25 (10.1)	Reference	
3´–UTR	CT	275 (47.2)	231 (43.8)	0.773 (0.512–1.168)	0.222	121 (43.4)	0.713 (0.440–1.157)	0.171	110 (44.4)	0.852 (0.502–1.444)	0.551
	TT	255 (43.7)	237 (45.0)	0.827 (0.547–1.250)	0.367	124 (44.4)	0.755 (0.465–1.224)	0.254	113 (45.6)	0.925 (0.545–1.569)	0.772
	C allele	381 (32.7)	349 (33.1)	Reference		189 (33.9)	Reference		160 (32.3)	Reference	
	T allele	785 (67.3)	705 (66.9)	0.966 (0.808–1.154)	0.701	369 (66.1)	0.932 (0.751–1.155)	0.519	336 (67.7)	1.007 (0.803–1.263)	0.950
HWE P value		0.082	0.81								
IFNG gene											
rs2430561	AA	413 (70.8)	371 (70.4)	Reference		196 (70.3)	Reference		175 (70.6)	Reference	
intron 1	AT	148 (25.4)	143 (27.1)	1.074 (0.819–1.409)	0.604	75 (26.9)	1.072 (0.773–1.487)	0.677	68 (27.4)	1.080 (0.769—1.516)	0.658
	TT	22 (3.8)	13 (2.5)	0.651 (0.322–1.317)	0.232	8 (2.9)	0.774 (0.337–1.777)	0.546	5 (2.0)	0.520 (0.193–1.401)	0.196
	A allele	974 (83.4)	885 (84.0)	Reference		467 (83.7)	Reference		418 (82.3)	Reference	
	T allele	192 (16.6)	169 (16.0)	0.966 (0.770–1.213)	0.766	91 (16.3)	0.993 (0.755–1.306)	0.961	78 (17.7)	0.938 (0.703–1.252)	0.664
HWE *P* value		0.062	0.859								
rs2069718	CC	21 (3.6)	13 (2.5)	Reference		9 (3.2)	Reference		4 (1.6)	Reference	
intron 3	CT	146 (25.0)	143 (27.1)	1.537 (0.738–3.202)	0.251	74 (26.5)	1.133 (0.492–2.610)	0.769	69 (27.8)	2.524 (0.831–7.669)	0.103
	TT	416 (71.4)	371 (70.4)	1.406 (0.691–2.862)	0.347	196 (70.3)	1.054 (0.472–2.354)	0.899	175 (70.6)	2.251 (0.759-6.682)	0.144
	C allele	188 (16.1)	169 (16.0)	Reference		92 (16.5)	Reference		77 (15.5)	Reference	
	T allele	978 (83.9)	885 (84.0)	1.005 (0.800–1.263)	0.964	466 (83.5)	0.965 (0.734–1.269)	0.798	419 (84.5)	1.051 (0.786–1.406)	0.736

Table 1 continued

SNP/location	Genotype/allele	Control	Case	Case versus control		MB versus control			PB versus control		
		No. (%)	No. (%)	OR (95% CI)*	*P* value*	No. (%)	OR (95% CI)	*P* value*	No. (%)	OR (95% CI)	*P* value*
HWE *P* value		0.074	0.859								

MB multibacillary leprosy, *PB* paucibacillary

* All data were calculated using the unconditional logistic regression, with an adjustment for sex

Chi-square test for deviation from the Hardy–Weinberg equilibrium (a value of $P < 0.001$ was regarded as a deviation from the HWE)

genotype CT of rs692527 in intron 5 of the *MRC1* gene had a significantly lower frequency in PB population (46.8%) than in the control population (51.8%; OR = 0.598, 95% CI [0.385–0.929], $P = 0.022$). In contrast, genotype TT of rs34856358 in intron 7 of the *MRC1* gene showed a higher frequency in PB population (19.8%) than in the control population (12.3%, OR = 1.688, 95% CI [1.080–2.638], $P = 0.022$). However, the allele frequencies of both SNPs rs692527 and rs34856358 were similar in the entire case and control populations (Table 1).

Alleles of rs3138557 of the *IFNG* gene were associated with leprosy subtypes

A total of 10 different alleles of the dinucleotide CA repeat (rs3138557) in the *IFNG* gene were identified in our samples (Table 2 and Figure S1). Allele CA_{12} had the highest frequency, followed by CA_{13}, CA_{14} and CA_{15}. We chose allele CA_{12} as the reference in the logistic regression analysis, with an adjustment for sex. As showed in Table 2, allele CA_{10} had a significantly higher frequency in leprosy patients (2.4 vs. 0.6% in controls, $P = 0.001$), especially in the MB patients (3.2%, $P < 0.001$), than in the control population. However, this allele had a considerably low frequency in both the cases and controls (<2.5%) and the observed significance should be treated with caution. Alleles CA_{13} and CA_{15} had a significantly higher frequencies in the MB population than in the control sample (CA_{13}, $P = 0.026$; CA_{15}, $P = 0.007$), whereas allele CA_{17} had a higher frequency in the PB population than in controls ($P = 0.040$). Similarly, allele CA_{17} also had a substantially low frequency in both the cases and controls (<2.5%), similar to that of CA_{10}. The overall genotype frequency of rs3138557 was similar in the cases and controls, with the exception of CA_{15}/CA_{15}, which had a significantly higher frequency in MB patients (7.9%) than the controls (5.3%) (Table S2).

Association of haplotypes of the *MRC1* gene and the *IFNG* gene with leprosy

We reconstructed haplotypes of the nine SNPs of the *MRC1* gene (rs2436680-rs2477637-rs2253120-rs692527-rs34301598-rs1926736-rs34856358-rs691461-rs691005) and of the three variants of the *IFNG* gene (rs2069718-rs2430561-rs3138557). A total of 64 haplotypes in the cases and 59 haplotypes in the controls were observed for the *MRC1* gene, whereas 18 haplotypes in the cases and 22 haplotypes in the controls were discerned for the *IFNG* gene. As three SNPs (rs2477637 and rs691461 of the *MRC1* gene and rs2069718 of the *IFNG* gene) in the two genes were in the same bins ($r^2 > 0.8$) with other proximal SNPs (Fig. 1), we excluded these three SNPs and

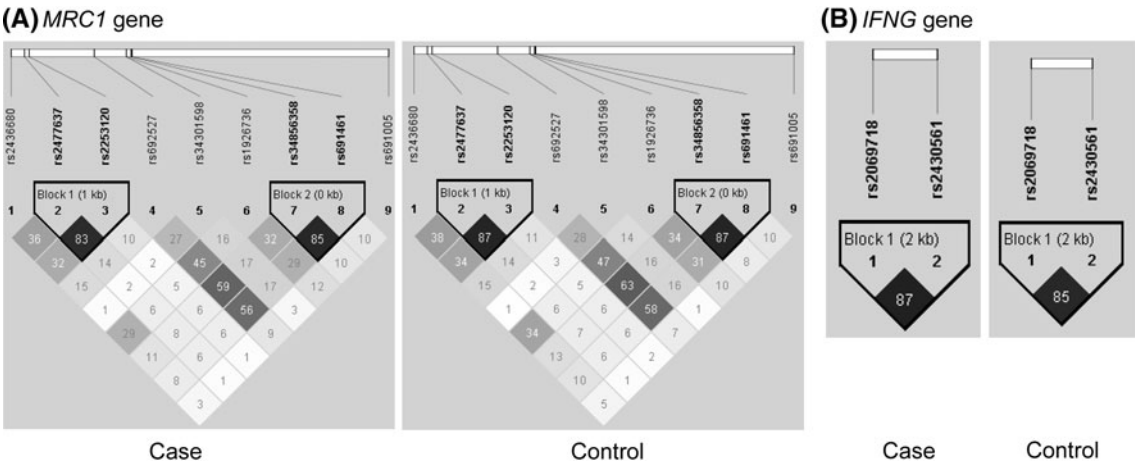

Fig. 1 The linkage disequilibrium (LD) structures of nine SNPs in the *MRC1* gene (**a**) and two SNPs in the *IFNG* gene (**b**) in leprosy patients and healthy controls from Yuxi, Yunnan Province of China. *Black squares* represent high LD as measured by r^2, gradually coloring down to *white squares* of low LD. The individual square showed the r^2 value for each SNP pair (r^2 value is multiplied by 100)

reconstructed haplotypes. We observed 43 haplotypes in the cases and 46 haplotypes in the controls for the *MRC1* gene and 14 haplotypes in the cases and 16 haplotypes in the controls for the *IFNG* gene. We grouped those haplotypes with a frequency lower than 3% in the case or control groups together and compared their distribution frequencies between the two groups (Table S3). The overall haplotype test was performed to show the global difference in haplotype frequencies between the case and control groups. There was no significant difference for the *MRC1* haplotypes between the two groups (Chi-square test: case vs. control, $P = 0.321$; MB vs. control, $P = 0.069$; PB vs. control, $P = 0.180$), but we observed a significant difference for the *IFNG* haplotypes (Chi-square test: case vs. control, $P = 0.007$; MB vs. control, $P < 0.001$; PB vs. control, $P = 0.208$). In particular, haplotype A-CA_{11} of the *IFNG* gene was found to be associated with leprosy (OR = 2.033, 95% CI [1.280–3.227], $P = 0.002$) and leprosy subtypes (MB, OR = 2.150, 95% CI [1.272–3.635], $P = 0.006$; PB, OR = 1.901, 95% CI [1.088–3.321], $P = 0.030$). However, this risk haplotype had a considerably low frequency (<5.0%) and the association should be treated with caution (Table S3).

Discussion

Accumulating evidence showed that host genetic background confers leprosy susceptibility and its clinical outcome (Alter et al. 2011; Cardoso et al. 2011; Mira 2006; Misch et al. 2010; Modlin 2010; Montoya and Modlin 2010). Based on genetic linkage scan of the genomes of affected families from South India, Siddiqui and coworkers (2001) identified a major leprosy susceptibility locus that is located on chromosome 10p13. Subsequent whole-genome scanning study of affected families from Vietnam by Mira et al. (2003) confirmed the involvement of this locus in paucibacillary leprosy; these authors further described a strong association of 6q25 with leprosy. Other loci, such as 6p21, 17q22, 20p13 (Miller et al. 2004), 20p12 (Tosh et al. 2002), 21q22 (Wallace et al. 2004), were also reported to be associated with leprosy or certain subtype of leprosy. Population-based case–control studies have identified a variety of SNPs in genes that were associated with leprosy, e.g. toll-like receptors (Bochud et al. 2008; Wong et al. 2010a), tumor necrosis factor-alpha (TNFα), mannose binding lectin (MBL), vitamin D receptor (VDR) (Sapkota et al. 2010), interleukin 10 (IL-10) (Malhotra et al. 2005), nucleotide-binding oligomerization domain containing 2 (NOD2) (Berrington et al. 2010). Recently, Zhang et al. (2009) performed the first GWAS for Chinese patients with leprosy and identified six genes (*CCDC122*, *C13orf31*, *NOD2*, *TNFSF15*, *HLA-DR*, and *RIPK2*) that showed association with resistance/susceptibility to leprosy. In particular, SNPs in the *CCDC122* and *C13orf31* genes have been replicated independently in patients from India and West Africa (Wong et al. 2010b), and *NOD2* variants were validated in patients from Nepal (Berrington et al. 2010). In a subsequent GWAS study, Zhang et al. (2011) identified two new loci at *IL23R* and *RAB32* that contribute to susceptibility to leprosy. However, we must confess that many of these genes and/or SNPs that were reported to be associated with leprosy were not well replicated in different populations. For instance, the *PARK2/PACRG* genes were suggested to be leprosy susceptible genes that were located in 6q25-q26 (Mira et al. 2003), but these genes were not found in the GWAS report (Zhang et al. 2009).

In this study, we genotyped 12 genetic variants in the *MRC1* gene and the *IFNG* gene, including rs1926736 of the *MRC1* gene and rs2430561 of the *IFNG* gene that were

Table 2 Allele frequencies of rs3138557 (CA repeat) of the *IFNG* gene in Han Chinese patients with leprosy patients and healthy controls from Yunnan, China

Allele		Control[a]	Case[a]	Case versus control		MB versus control			PB versus control		
Size (bp)	No. of repeats	No. (%)	No. (%)	OR (95% CI)*	*P* value*	No. (%)	OR (95% CI)	*P* value*	No.(%)	OR (95% CI)	*P* value*
124	12	350 (30.2)	294 (28.1)	Reference[c]		141 (25.5)	Reference		153 (31.0)	Reference	
120	10	7 (0.6)	25 (2.4)	4.202 (1.785–9.894)	0.001	18 (3.2)	3.898 (1.469–10.346)	0.000	7 (1.4)	2.120 (0.728–6.177)	0.168
122	11	108 (9.3)	104 (9.9)	1.135 (0.830–1.552)	0.426	58 (10.5)	1.439 (0.976–2.124)	0.173	46 (9.3)	0.965 (0.649–1.433)	0.858
126	13	215 (18.6)	218 (20.8)	1.232 (0.964–1.576)	0.096	118 (21.3)	1.435 (0.899–2.289)	0.026	100 (20.2)	1.095 (0.806–1.487)	0.562
128	14	219 (18.9)	165 (15.7)	0.891 (0.689–1.151)	0.375	72 (13.0)	0.913 (0.640–1.303)	0.222	93 (18.8)	0.957 (0.702–1.304)	0.781
130	15	208 (18.0)	195 (18.6)	1.163 (0.904–1.496)	0.240	121 (21.8)	1.369 (0.713–2.631)	0.007	74 (15.0)	0.870 (0.626–1.209)	0.407
132	16	20 (1.7)	11 (1.0)	0.689 (0.323–1.469)	0.335	6 (1.1)	0.828 (0.164–4.171)	0.631	5 (1.0)	0.594 (0.218–1.619)	0.308
134	17	14 (1.2)	24 (2.3)	1.934 (0.979–3.820)	0.057	10 (1.8)	0.473 (0.158–1.413)	0.237	14 (2.8)	2.239 (1.037–4.833)	0.040
136[b]	18[b]	17 (1.5)	12 (1.1)	0.840 (0.393–1.797)	0.654	10 (1.8)	2.481 (0.492–12.502)	0.346	2 (0.4)	0.281 (0.064–1.235)	0.093

* All data were calculated using the unconditional logistic regression and were adjusted for sex

[a] Three case samples and four control samples were failed to be genotyped

[b] Including one control sample with 19 CA repeats that had a size of 138 bp

[c] Samples with the major allele were used as the reference

reported to be associated with leprosy (Alter et al. 2010; Cardoso et al. 2010) in Han Chinese from Southwest China. We attempted to answer two questions: 1. Can the reported association of rs1926736 and rs2430561 with leprosy be validated in independent population from Southwest China? 2. Are there any other risk alleles in these two genes and influence the susceptibility to leprosy?

According to the estimation for statistical power of the test, the current sample size (527 patients and 583 controls) had a sufficient power to identify risk allele supposing an OR value of 1.6. Moreover, the matrilineal genetic structures of the case and control populations were very similar (authors' unpublished data), suggesting that there was no potential population stratification and sampling bias for the two populations under study. Unfortunately, with these two well-matched case and control populations, we found no evidence for a significant association of SNPs rs1926736 and rs2430561 with leprosy in patients from Yunnan, China. The failure to validate the previously reported associations (Alter et al. 2010; Cardoso et al. 2010) was unexpected, especially when we considered the fact that rs1926736 of the *MRC1* gene was initially identified in leprosy patients from Vietnam (Alter et al. 2010), which is proximal to Yunnan, China. A comparison of MAF of rs1926736 in our samples and those from the HapMap datasets showed that this allele presented a marvelously regional difference which might account for the discrepancy between different studies. Intriguingly, we found that genotypes of two SNPs (rs692527 and rs34856358) in the intron region of the *MRC1* gene were associated with PB, and alleles of the dinucleotide CA repeat (rs3138557) in the *IFNG* gene were associated with leprosy, particularly for MB (Table 1). Note that these positive associations should be received with caution, as the statistical power was found to be low given the estimated OR values for each SNP (Table 1).

MRC1 is a member of the C-type lectin receptor family which encodes the human mannose receptor (MR). As one of the pattern recognition receptors, MR can recognize a wide range of microorganisms so that phagocytes can uptake microbial components and other antigenic particles during the early event of infection (East and Isacke 2002). The *MRC1* gene played an active role in innate and adaptive immunity and was naturally proposed to be a candidate gene at the chromosomal region 10p13 that was associated with leprosy (Mira et al. 2003; Siddiqui et al. 2001). Genetic variants of the *MRC1* gene have also been reported to confer susceptibility to increased risk of sarcoidosis (Hattori et al. 2010). Despite that we failed to validate the reported association of rs1926736 (p.G396S) of the *MRC1* gene with leprosy (Alter et al. 2010), we identified two other SNPs in the intron region of this gene that confer a susceptibility to leprosy. This observation

suggested that the *MRC1* gene might be actively involved in leprosy. Further studies should be carried out to elucidate the exact role of the *MRC1* gene in this disease.

IFN-γ is a multifunctional cytokine that plays a crucial role in immune response against intracellular infection. In human beings, the IL12-23/IFN-γ axis is crucial for protective immunity to mycobacterial infection and has been frequently selected as candidate genes/pathway in the study of mycobacterial disease (Al-Muhsen and Casanova 2008; Cardoso et al. 2011). SNPs rs2430561 and rs3138557 in the first intron of the *IFNG* gene were reported to be associated with IFN-γ production in several diseases including leprosy (Awad et al. 1999; Cardoso et al. 2010; Pravica et al. 1999; Rossouw et al. 2003). Specifically, allele T of rs2430561 creates an NF-κB binding site (Pravica et al. 2000) and allele CA_{12} of the *IFNG* gene contributes to the highest IFN-γ expression (Pravica et al. 1999). Allele T of rs2430561 had a much lower frequency in our samples (<17.0%) than in Brazilians (>30%) (Cardoso et al. 2010) and South Africans (>24%) (Rossouw et al. 2003), but the T allele frequency was similar in our leprosy patients and controls, showing no association with leprosy or its subtypes. The finding for association of four different alleles of rs3138557 in the *IFNG* gene with leprosy, especially with MB leprosy, was a little unexpected, as these alleles contained different CA repeats and there was no direct correlation of the number of CA repeats with the risk. Nonetheless, the association of rs3138557 with leprosy would be compatible with the various immune responses contributed by IFNG during the onset of leprosy (Modlin 2010; Montoya and Modlin 2010). We speculated that risk alleles of rs3138557 and risk haplotype of the *IFNG* gene might have a greater influence on IFN-γ expression. The risk haplotype A-CA_{11} of the *IFNG* gene that we found, to some extent, supported the result of a previous meta-analysis that +874 T allele was associated with higher IFN-γ production and resistance to mycobacterial infection (Pacheco et al. 2008).

In summary, we genotyped 12 genetic variants in the *MRC1* gene and the *IFNG* gene to discern their potential association with leprosy in Han Chinese. We found no support for the reported association between SNPs rs1926736 and rs2430561 and leprosy. However, we found that two SNPs in the intron region of the *MRC1* gene were associated with paucibacillary leprosy, and four different alleles of rs3138557 and haployte of the *IFNG* gene were associated with leprosy and/or leprosy subtypes in Han Chinese from Southwest China. Further studies, particularly functional assays, such as in vitro IFNG release, phagocytosis, in those genotyped leprosy patients and healthy Chinese, will be essential to clarify the exact role of these two genes in leprosy.

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References

Alcaïs A, Mira M, Casanova JL, Schurr E, Abel L (2005) Genetic dissection of immunity in leprosy. Curr Opin Immunol 17:44–48

Al-Muhsen S, Casanova JL (2008) The genetic heterogeneity of mendelian susceptibility to mycobacterial diseases. J Allergy Clin Immunol 122:1043–1051

Alter A, de Léséleuc L, Van Thuc N, Thai VH, Huong NT, Ba NN, Cardoso CC, Grant AV, Abel L, Moraes MO, Alcaïs A, Schurr E (2010) Genetic and functional analysis of common MRC1 exon 7 polymorphisms in leprosy susceptibility. Hum Genet 127:337–348

Alter A, Grant A, Abel L, Alcaïs A, Schurr E (2011) Leprosy as a genetic disease. Mamm Genome 22:19–31

Awad M, Pravica V, Perrey C, El Gamel A, Yonan N, Sinnott PJ, Hutchinson IV (1999) CA repeat allele polymorphism in the first intron of the human interferon-gamma gene is associated with lung allograft fibrosis. Hum Immunol 60:343–346

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265

Berrington WR, Macdonald M, Khadge S, Sapkota BR, Janer M, Hagge DA, Kaplan G, Hawn TR (2010) Common polymorphisms in the NOD2 gene region are associated with leprosy and its reactive states. J Infect Dis 201:1422–1435

Bochud PY, Hawn TR, Siddiqui MR, Saunderson P, Britton S, Abraham I, Argaw AT, Janer M, Zhao LP, Kaplan G, Aderem A (2008) Toll-like receptor 2 (TLR2) polymorphisms are associated with reversal reaction in leprosy. J Infect Dis 197:253–261

Britton WJ, Lockwood DN (2004) Leprosy. Lancet 363:1209–1219

Cardoso CC, Pereira AC, Brito-de-Souza VN, Dias-Baptista IM, Maniero VC, Venturini J, Vilani-Moreno FR, de Souza FC, Ribeiro-Alves M, Sarno EN, Pacheco AG, Moraes MO (2010) IFNG +874 T>A single nucleotide polymorphism is associated with leprosy among Brazilians. Hum Genet 128:481–490

Cardoso CC, Pereira AC, de Sales Marques C, Moraes MO (2011) Leprosy susceptibility: genetic variations regulate innate and adaptive immunity, and disease outcome. Future Microbiol 6:533–549

Cole ST, Eiglmeier K, Parkhill J, James KD, Thomson NR, Wheeler PR, Honoré N, Garnier T, Churcher C, Harris D, Mungall K, Basham D, Brown D, Chillingworth T, Connor R, Davies RM, Devlin K, Duthoy S, Feltwell T, Fraser A, Hamlin N, Holroyd S, Hornsby T, Jagels K, Lacroix C, Maclean J, Moule S, Murphy L, Oliver K, Quail MA, Rajandream MA, Rutherford KM, Rutter S, Seeger K, Simon S, Simmonds M, Skelton J, Squares R, Squares S, Stevens K, Taylor K, Whitehead S, Woodward JR, Barrell BG (2001) Massive gene decay in the leprosy bacillus. Nature 409:1007–1011

East L, Isacke CM (2002) The mannose receptor family. Biochim Biophys Acta 1572:364–386

Gauderman WJ (2002) Sample size requirements for matched case-control studies of gene-environment interaction. Stat Med 21:35–50

Hattori T, Konno S, Takahashi A, Isada A, Shimizu K, Taniguchi N, Gao P, Yamaguchi E, Hizawa N, Huang SK, Nishimura M (2010) Genetic variants in mannose receptor gene (MRC1) confer susceptibility to increased risk of sarcoidosis. BMC Med Genet 11:151

Holland MM, Parson W (2011) GeneMarker® HID: A reliable software tool for the analysis of forensic STR data. J Forensic Sci 56:29–35

Khani-Hanjani A, Lacaille D, Hoar D, Chalmers A, Horsman D, Anderson M, Balshaw R, Keown PA (2000) Association between dinucleotide repeat in non-coding region of interferon-gamma gene and susceptibility to, and severity of, rheumatoid arthritis. Lancet 356:820–825

Li Y-Y, Li X-A, He L, Wang D, Chen W-Y, Chen L, Lu J-B, Yao Y-G (2011) Trends in new leprosy case detection over 57 years (1952–2008) in Yuxi, Yunnan Province of Southwest China. Lepr Rev 82:6–16

Malhotra D, Darvishi K, Sood S, Sharma S, Grover C, Relhan V, Reddy BS, Bamezai RN (2005) IL-10 promoter single nucleotide polymorphisms are significantly associated with resistance to leprosy. Hum Genet 118:295–300

Miller EN, Jamieson SE, Joberty C, Fakiola M, Hudson D, Peacock CS, Cordell HJ, Shaw MA, Lins-Lainson Z, Shaw JJ, Ramos F, Silveira F, Blackwell JM (2004) Genome-wide scans for leprosy and tuberculosis susceptibility genes in Brazilians. Genes Immun 5:63–67

Mira MT (2006) Genetic host resistance and susceptibility to leprosy. Microbes Infect 8:1124–1131

Mira MT, Alcaïs A, Van Thuc N, Thai VH, Huong NT, Ba NN, Verner A, Hudson TJ, Abel L, Schurr E (2003) Chromosome 6q25 is linked to susceptibility to leprosy in a Vietnamese population. Nat Genet 33:412–415

Misch EA, Berrington WR, Vary JC Jr, Hawn TR (2010) Leprosy and the human genome. Microbiol Mol Biol Rev 74:589–620

Modlin RL (2010) The innate immune response in leprosy. Curr Opin Immunol 22:48–54

Monot M, Honoré N, Garnier T, Zidane N, Sherafi D, Paniz-Mondolfi A, Matsuoka M, Taylor GM, Donoghue HD, Bouwman A, Mays S, Watson C, Lockwood D, Khamesipour A, Dowlati Y, Jianping S, Rea TH, Vera-Cabrera L, Stefani MM, Banu S, Macdonald M, Sapkota BR, Spencer JS, Thomas J, Harshman K, Singh P, Busso P, Gattiker A, Rougemont J, Brennan PJ, Cole ST (2009) Comparative genomic and phylogeographic analysis of *Mycobacterium leprae*. Nat Genet 41:1282–1289

Montoya D, Modlin RL (2010) Learning from leprosy: insight into the human innate immune response. Adv Immunol 105:1–24

Pacheco AG, Cardoso CC, Moraes MO (2008) IFNG+874T/A, IL10-1082G/A and TNF-308G/A polymorphisms in association with tuberculosis susceptibility: a meta-analysis study. Hum Genet 123:477–484

Pravica V, Asderakis A, Perrey C, Hajeer A, Sinnott PJ, Hutchinson IV (1999) In vitro production of IFN-gamma correlates with CA repeat polymorphism in the human IFN-gamma gene. Eur J Immunogenet 26:1–3

Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV (2000) A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. Hum Immunol 61:863–866

Rossouw M, Nel HJ, Cooke GS, van Helden PD, Hoal EG (2003) Association between tuberculosis and a polymorphic NFkappaB binding site in the interferon gamma gene. Lancet 361:1871–1872

Sapkota BR, Macdonald M, Berrington WR, Misch EA, Ranjit C, Siddiqui MR, Kaplan G, Hawn TR (2010) Association of TNF, MBL, and VDR polymorphisms with leprosy phenotypes. Hum Immunol 71:992–998

Siddiqui MR, Meisner S, Tosh K, Balakrishnan K, Ghei S, Fisher SE, Golding M, Shanker Narayan NP, Sitaraman T, Sengupta U, Pitchappan R, Hill AV (2001) A major susceptibility locus for leprosy in India maps to chromosome 10p13. Nat Genet 27:439–441

Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 68:978–989

Tosh K, Meisner S, Siddiqui MR, Balakrishnan K, Ghei S, Golding M, Sengupta U, Pitchappan RM, Hill AV (2002) A region of chromosome 20 is linked to leprosy susceptibility in a South Indian population. J Infect Dis 186:1190–1193

Wallace C, Fitness J, Hennig B, Sichali L, Mwaungulu L, Pönnighaus JM, Warndorff DK, Clayton D, Fine PE, Hill AV (2004) Linkage analysis of susceptibility to leprosy type using an IBD regression method. Genes Immun 5:221–225

WHO (2010) Global leprosy situation, 2010. Wkly Epidemiol Rec 85:337–348

Wong SH, Gochhait S, Malhotra D, Pettersson FH, Teo YY, Khor CC, Rautanen A, Chapman SJ, Mills TC, Srivastava A, Rudko A, Freidin MB, Puzyrev VP, Ali S, Aggarwal S, Chopra R, Reddy BS, Garg VK, Roy S, Meisner S, Hazra SK, Saha B, Floyd S, Keating BJ, Kim C, Fairfax BP, Knight JC, Hill PC, Adegbola RA, Hakonarson H, Fine PE, Pitchappan RM, Bamezai RN, Hill AV, Vannberg FO (2010a) Leprosy and the adaptation of human toll-like receptor 1. PLoS Pathog 6: e1000979

Wong SH, Hill AV, Vannberg FO (2010b) Genomewide association study of leprosy. N Engl J Med 362:1446–1447

Zhang FR, Huang W, Chen SM, Sun LD, Liu H, Li Y, Cui Y, Yan XX, Yang HT, Yang RD, Chu TS, Zhang C, Zhang L, Han JW, Yu GQ, Quan C, Yu YX, Zhang Z, Shi BQ, Zhang LH, Cheng H, Wang CY, Lin Y, Zheng HF, Fu XA, Zuo XB, Wang Q, Long H, Sun YP, Cheng YL, Tian HQ, Zhou FS, Liu HX, Lu WS, He SM, Du WL, Shen M, Jin QY, Wang Y, Low HQ, Erwin T, Yang NH, Li JY, Zhao X, Jiao YL, Mao LG, Yin G, Jiang ZX, Wang XD, Yu JP, Hu ZH, Gong CH, Liu YQ, Liu RY, Wang DM, Wei D, Liu JX, Cao WK, Cao HZ, Li YP, Yan WG, Wei SY, Wang KJ, Hibberd ML, Yang S, Zhang XJ, Liu JJ (2009) Genomewide association study of leprosy. N Engl J Med 361:2609–2618

Zhang F, Liu H, Chen S, Low H, Sun L, Cui Y, Chu T, Li Y, Fu X, Yu Y, Yu G, Shi B, Tian H, Liu D, Yu X, Li J, Lu N, Bao F, Yuan C, Liu J, Zhang L, Sun Y, Chen M, Yang Q, Yang H, Yang R, Wang Q, Zuo F, Zhang H, Khor CC, Hibberd ML, Yang S, Zhang X (2011) Identification of two new loci at IL23R and RAB32 that influence susceptibility to leprosy. Nat Genet 43:1247–1251

Online Supplementary Materials

There are three supplementary tables and one supplementary figure.

Table S1 Primers for genotyping 8 SNPs by using SNaPshot assay

SNP ID	Primer (5' - 3')	
rs2436680	Forward	AATATTCAGCATACCTGTAATCATTAATC
	Reverse	GGAGCATAAATAATCTCTCAACAAA
	Probe	T$(GACT)_1$GATAAATAATTTATCTGGGTTACTAGTTAAGATGC
rs691461	Forward	GGTTTTCTAATTTATTTACTAATTCTCAAGC
	Reverse	AACTGGAGTGCACATTTAACTCTAC
	Probe	CT$(GACT)_2$AAAGCAACTTTGGCCATCCAATTTCCTAAAATAAT
rs2477637	Forward	AAAAAAGGATCTGGTAAGCATTT
	Reverse	GATGTGTTTAATTTATGTTTATGTCACA
	Probe	ACT$(GACT)_5$CCTTTAATTAAATCAAAATTGAGTTCA
rs2253120	Forward	AAAAGTGTGTCATTTTTGCACTC
	Reverse	AATCTCAGATTATGAGTGTTGCATT
	Probe	$(GACT)_5$GAGTCACAGGCATAGAGAGTGATAGCAACCCAGTC
rs692527	Forward	ACAACATCTGCTTTTGAATATAGTAC
	Reverse	AGGATTCTCACACAAAACAATAAAG
	Probe	T$(GACT)_6$ATATAGTACCCAACACATCAGGGATACTCTGAGAA
rs691005	Forward	GTTTGAAGGTATTAATCCTCAGTATTCT
	Reverse	CATTCTACATCAGTGAATTTACCAAC
	Probe	CT$(GACT)_7$GTATTCTCTCTTTGGTACAACATAGTAAATCTCTC
rs2069718	Forward	AAATGTGGTGAGTAGCCATAGTG
	Reverse	AAATTGAACTACTTGCATCTCCTC
	Probe	ACT$(GACT)_8$ATGGCAGAGCCAAGAGGAAGGTAAATGGTCCACAT
rs34856358	Forward	ATCCTAACTAACCTGTTTTCTGCT
	Reverse	AATCAGAACTGGTATGTCTGAATAAC
	Probe	$(GACT)_{11}$CTGCTAAATCATTTGCAAACTTTACTGGCTA

(GACT)n, n repeats of “GACT”

Table S2 Genotype frequencies of rs3138557 (CA repeat) of the *IFNG* gene in Han Chinese with and without leprosy from Yunnan

Genotype	Control [a]	Case [a]	Case vs. Control		MB vs. Control			PB vs. Control		
	No. (%)	No. (%)	OR (95% CI) *	*P* value*	No. (%)	OR (95% CI)	*P* value	No. (%)	OR (95% CI)	*P* value
12&14	94 (16.1)	74 (14.0)	reference [#]		33 (11.8)	reference		41 (16.5)	reference	
11&11	12 (2.1)	15 (2.8)	1.613 (0.707-3.676)	0.256	9 (3.2)	2.055 (0.790-5.343)	0.140	6 (2.4)	1.206 (0.420-3.462)	0.728
11&12	33 (5.7)	27 (5.1)	1.060 (0.584-1.927)	0.848	16 (5.7)	1.416 (0.689-2.912)	0.344	11 (4.4)	0.780 (0.358-1.700)	0.531
11&14	37 (6.3)	22 (4.2)	0.717 (0.389-1.324)	0.288	10 (3.6)	0.743 (0.332-1.663)	0.469	12 (4.8)	0.693 (0.327-1.469)	0.339
12&12	64 (11.0)	50 (9.5)	0.993 (0.613-1.608)	0.977	23 (8.2)	1.026 (0.550-1.912)	0.936	27 (10.9)	0.951 (0.530-1.704)	0.865
12&13	41 (7.0)	38 (7.2)	1.261 (0.734-2.168)	0.401	19 (6.8)	1.441 (0.730-2.842)	0.292	19 (7.7)	1.113 (0.575-2.157)	0.750
12&15	43 (7.4)	38 (7.2)	1.164 (0.681-1.991)	0.578	23 (8.2)	1.582 (0.828-3.022)	0.165	15 (6.0)	0.848 (0.422-1.705)	0.645
13&13	36 (6.2)	38 (7.2)	1.359 (0.782-2.361)	0.276	19 (6.8)	1.558 (0.784-3.097)	0.206	19 (7.7)	1.233 (0.631-2.410)	0.540
13&15	74 (12.7)	66 (12.5)	1.205 (0.765-1.900)	0.421	37 (13.3)	1.535 (0.873-2.700)	0.137	29 (11.7)	0.967 (0.547-1.709)	0.907
14&14	29 (5.0)	25 (4.7)	1.162 (0.624-2.162)	0.636	11 (3.9)	1.161 (0.519-2.597)	0.716	14 (5.6)	1.125 (0.537-2.360)	0.755
15&15	31 (5.3)	34 (6.5)	1.518 (0.849-2.713)	0.159	22 (7.9)	2.224 (1.124-4.402)	0.022	13 (5.2)	0.994 (0.461-2.145)	0.988
15&18	10 (1.7)	10 (1.9)	1.254 (0.493-3.192)	0.634	8 (2.9)	2.294 (0.830-6.342)	0.110	2 (0.8)	0.464 (0.097-2.225)	0.337
Others [b]	75 (12.9)	87 (16.5)	1.490 (0.962-2.306)	0.074	47 (16.8)	1.822 (1.060-3.133)	0.030	40 (16.1)	1.237 (0.725-2.110)	0.436

* All data were calculated by using the unconditional logistic regression, with an adjustment for sex.

Samples with the major allele were used as the reference.

[a] Excluding 3 case samples and 4 control samples that were not successfully genotyped.

[b] Including one control sample with 19 CA repeats.

Table S3 Association of the *MRC1* and *IFNG* haplotypes with leprosy in Han Chinese

Haplotype [a]	Control	Case	Case vs. Control		MB vs. Control			PB vs. Control		
	No. (%)	No. (%)	OR (95% CI) *	*P* value *	No. (%)	OR (95% CI)	*P* value	No. (%)	OR (95% CI)	*P* value
MRC1 gene										
CCCGCTT	69 (5.9)	76 (7.2)	1.235 (0.882-1.731)	0.229	48 (8.6)	1.496 (1.020-2.195)	0.041	28 (5.6)	0.951 (0.605-1.495)	0.909
CCTACCT	88 (7.5)	74 (7.0)	0.925 (0.671-1.275)	0.683	28 (5.0)	0.647 (0.418-1.003)	0.051	46 (9.3)	1.252 (0.862-1.819)	0.238
CTCACTT	145 (12.4)	135 (12.8)	1.034 (0.805-1.329)	0.798	62 (11.1)	0.880 (0.642-1.207)	0.476	73 (14.7)	1.215 (0.897-1.646)	0.205
TCCACTT	43 (3.7)	30 (2.8)	0.765 (0.476-1.229)	0.285	16 (2.9)	0.771 (0.430-1.381)	0.479	14 (2.8)	0.759 (0.411-1.400)	0.462
TCCGCTT	58 (5.0)	52 (4.9)	0.991 (0.675-1.455)	1.000	21 (3.8)	0.747 (0.449-1.224)	0.324	31 (6.2)	1.274 (0.813-1.996)	0.286
TCTATCC	252 (21.6)	228 (21.6)	1.001 (0.818-1.226)	1.000	125 (22.4)	1.047 (0.821-1.335)	0.709	103 (20.8)	0.951 (0.734-1.230)	0.744
TCTATCT	181 (15.5)	131 (12.4)	0.772 (0.606-0.984)	0.038	77 (13.8)	0.871 (0.653-1.162)	0.387	54 (10.9)	0.665 (0.481-0.919)	0.014
Other [b]	330 (28.3)	328 (31.1)	1.145 (0.954-1.373)	0.149	181 (32.4)	1.216 (0.978-1.513)	0.081	147 (29.6)	1.067 (0.847-1.344)	0.596
IFNG gene [c]										
A-CA_{11}	29 (2.5)	52 (4.9)	2.033 (1.280-3.227)	0.002	29 (5.2)	2.150 (1.272-3.635)	0.006	23 (4.6)	1.901 (1.088-3.321)	0.030
A-CA_{12}	254 (21.8)	206 (19.5)	0.871 (0.708-1.070)	0.190	97 (17.4)	0.755 (0.583-0.979)	0.035	109 (22.0)	1.008 (0.781-1.299)	0.948
A-CA_{13}	212 (18.2)	215 (20.4)	1.152 (0.932-1.423)	0.196	117 (21.0)	1.195 (0.928-1.538)	0.169	98 (19.8)	1.104 (0.846-1.442)	0.491
A-CA_{14}	216 (18.5)	165 (15.7)	0.815 (0.652-1.018)	0.071	72 (12.9)	0.651 (0.488-0.869)	0.004	93 (18.8)	1.011 (0.772-1.325)	0.945
A-CA_{15}	203 (17.4)	194 (18.4)	1.069 (0.860-1.328)	0.579	120 (21.5)	1.301 (1.010-1.675)	0.047	74 (14.9)	0.829 (0.620-1.108)	0.222
T-CA_{11}	79 (6.8)	52 (4.9)	0.713 (0.497-1.023)	0.071	29 (5.2)	0.754 (0.487-1.169)	0.242	23 (4.6)	0.667 (0.414-1.074)	0.117
T-CA_{12}	96 (8.2)	88 (8.3)	1.014 (0.750-1.372)	0.939	44 (7.9)	0.954 (0.658-1.385)	0.851	44 (8.9)	1.082 (0.745-1.571)	0.700
Other [b]	69 (5.9)	76 (7.2)	1.234 (0.881-1.729)	0.229	46 (8.2)	1.429 (0.970-2.016)	0.079	30 (6.0)	1.020 (0.656-1.588)	0.910

* All data were calculated by using the Fisher's exact test.

[a] The order of SNPs in each haplotype for the *MRC1* gene is rs2436680-rs2253120-rs692527-rs34301598-rs1926736-rs34856358-rs691005. The order of SNPs in haplotypes for the *IFNG* gene is rs2430561-rs3138557. We excluded rs2477637 and rs691461 of the *MRC1* gene and rs2069718 of the *IFNG* gene that were in the same bins ($r^2 > 0.8$) with other proximal SNPs (cf. Figure 1).
[b] Haplotypes with a frequency lower than 3% in the case or control groups were aggregated together.
[c] Excluding 3 case samples and 4 control samples that were not successfully genotyped rs3138557 (CA repeats).

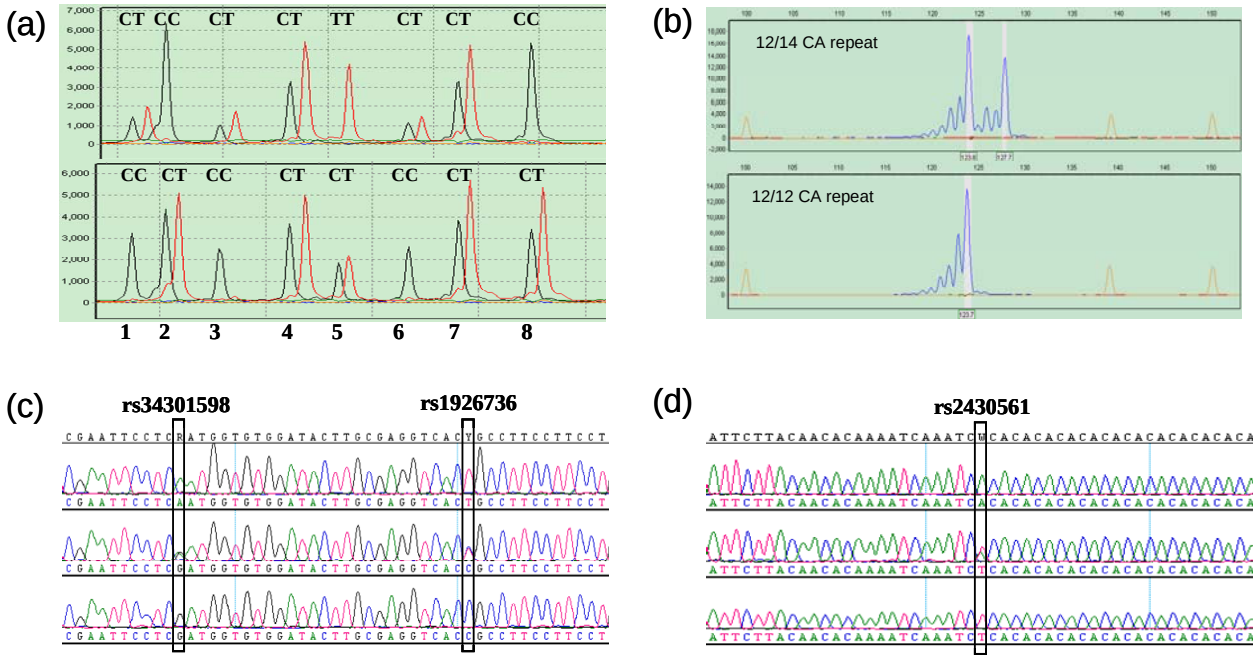

Figure S1 Genotyping results for genetic variants of the *MRC1* and *IFNG* gene. (a) SNaPshot profile of eight SNPs (1, rs2436680; 2, rs691461; 3, rs2477637; 4, rs2253120; 5, rs692527; 6, rs691005; 7, rs2069718; 8, rs34856358). (b) Variant rs3138557 (CA repeat) was genotyped by using GeneScan. (c) and (d) showed sequencing electropherograms of rs34301598, rs1926736 and rs2430561.