Missense Variants in *HIF1A* and *LACC1* Contribute to Leprosy Risk in Han Chinese

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Genome-wide association studies (GWASs) and genome-wide linkage studies (GWLSs) have identified numerous risk genes affecting the susceptibility to leprosy. However, most of the reported GWAS hits are noncoding variants and account for only part of the estimated heritability for this disease. In order to identify additional risk genes and map the potentially functional variants within the GWAS loci, we performed a three-stage study combining whole-exome sequencing (WES; discovery stage), targeted next-generation sequencing (NGS; screening stage), and refined validation of risk missense variants in 1,433 individuals with leprosy and 1,625 healthy control individuals from Yunnan Province, Southwest China. We identified and validated a rare damaging variant, rs142179458 (c.1045G>A [p.Asp349Asn]) in *HIF1A*, as contributing to leprosy risk ($p = 4.95 \times 10^{-9}$, odds ratio [OR] = 2.266). We were able to show that affected individuals harboring the risk allele presented with multibacillary leprosy at an earlier age ($p = 0.025$). We also confirmed the association between missense variant rs3764147 (c.760A>G [p.Ile254Val]) in the GWAS hit *LACC1* (formerly *C13orf31*) and leprosy ($p = 6.11 \times 10^{-18}$, OR = 1.605). By using the population attributable fraction, we have shown that *HIF1A* and *LACC1* are the major genes with missense variants contributing to leprosy risk in our study groups. Consistently, mRNA expression levels of both *HIF1A* and *LACC1* were upregulated in the skin lesions of individuals with leprosy and in *Mycobacterium leprae*-stimulated cells, indicating an active role of *HIF1A* and *LACC1* in leprosy pathogenesis.

Introduction

Leprosy (MIM: 609888) is a chronic infectious and neurological disease caused by *Mycobacterium leprae* (*M. leprae*) and has an ancient history.[1–3] There are around 200,000 new cases each year worldwide despite the fact that most countries have achieved the World Health Organization (WHO) leprosy elimination criterion (less than one case per population of 10,000).[4] Leprosy manifests in five clinical forms, including tuberculoid (TT), borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL), and lepromatous (LL).[5] For treatment purposes, the WHO has categorized leprosy as multibacillary (MB, including LL, BL, and BB) or paucibacillary (PB, including TT and BT).[6]

Previous genetic studies using a family-based association analysis,[7,8] candidate-gene strategy,[9–13] or genome-wide association study (GWAS)[14–19] have identified a variety of risk loci or susceptibility genes for leprosy. These risk genes—such as *NOD2* (MIM: 605956), *PRKN* (formerly *PARK2* [MIM: 602544]), *LRRK2* (MIM: 609007), *APOE* (MIM: 107741), *PINK1* (formerly *PARK6* [MIM: 608309]), and *PARL* (MIM: 607858)—are involved in the innate and adaptive immune systems, neurological pathways, and mitochondrion-related pathways.[10,11,20–23] The large-scale GWAS analysis of Chinese populations in the last decade greatly broadened our knowledge regarding the genetic susceptibility to leprosy[14–16,18,19] and hypersensitivity to dapsone treatment.[24] However, the effect size of the array-based GWAS hit variants was modest and accounted for only around 13.53% of the genetic heritability of leprosy.[16] Additional genes and variants with stronger effect sizes need to be identified to address the so-called "missing heritability."[25,26] Moreover, most SNPs in the GWAS loci were located in noncoding regions with unknown function, and the functional variants and causal genes underlying the GWAS loci remain to be recognized. The recent utilization of next-generation sequencing (NGS) technologies, such as whole-exome sequencing (WES) and targeted NGS, can be very powerful for the identification of rare potentially damaging variants contributing to disease.[27,28]

In this study, we aimed to identify protein-coding variants and risk genes that might have a large effect on leprosy susceptibility by using NGS technology and independent validation. Using our data, we were also able to fine-map the potentially functional variants and genes within the GWAS and genome-wide linkage study (GWLS) loci. We found that a rare missense variant in *HIF1A* (MIM: 603348) and a common missense variant in *LACC1* (formerly *C13orf31* [MIM: 613409]) contribute to leprosy susceptibility in Han Chinese.

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Table 1. Clinical and Demographic Information of Individuals with Leprosy and Healthy Control Individuals from Yunnan Province, Southwest China

	WES[a]	Targeted NGS[b]	Replication
Individuals with Leprosy			
Total number	108	798	527
Age range in years (mean ± SD)	12–92 (56.8 ± 16.0)	8–100 (56.2 ± 14.4)	16–97 (61.0 ± 12.5)
Onset age range in years (mean ± SD)	5–73 (25.3 ± 12.7)	4–87 (26.5 ± 12.5)	2–67 (24.8 ± 12.4)
No. of females	42 (38.9%)	251 (31.5%)	140 (26.6%)
No. of MB individuals	53 (49.1%)	452 (59.2%)	279 (52.9%)
Control Individuals			
Total number	52	990	583
Age (year, range, mean ± SD)	13–67 (43.1 ± 13.5)	9–83 (38.1 ± 14.0)	4–88 (36.0 ± 15.5)
No. of females	23 (44.2%)	439 (44.3%)	219 (37.6%)

Abbreviations are as follow: WES, samples were analyzed by whole-exome sequencing; targeted NGS, samples were analyzed by targeted next-generation sequencing technologies; replication, the Yuxi sample was used for replication; MB, multibacillary leprosy.[a]The WES group contained 53 individuals with lepromatous (LL) leprosy, 55 individuals with tuberculoid (TT) leprosy, and 52 matched healthy control individuals from the Wenshan Prefecture.
[b]35 individuals with leprosy had missing information regarding MB and PB classification and were thus excluded from the percentages of MB individuals.

Material and Methods

Subjects

A total of 3,058 individuals with or without leprosy were analyzed in the three-stage analysis (Table 1 and Figure 1). In the WES discovery stage (sample I), 108 unrelated individuals with leprosy from both poles of the clinical spectrum (53 LL and 55 TT) and with a positive family history (each family had at least two related members with leprosy) were analyzed. A further 52 unaffected individuals from the same villages as the affected individuals were also enrolled in the study. All of these subjects were genetically unrelated and were from the Wenshan Prefecture, Yunnan Province, Southwest China. In the screening stage (sample II), 798 individuals with leprosy and 990 healthy subjects from the Wenshan Prefecture were analyzed by targeted NGS. In the replication cohort (sample III), we used the previously reported case and control cohort (527 individuals with leprosy and 583 healthy subjects) from the Yuxi Prefecture, Yunnan Province.[22,23,29–31] All individuals with leprosy were diagnosed by clinical and histopathological features and/or bacteriological index (if available), as had been described in our previous epidemiological study.[32] The regionally matched healthy individuals had no history of leprosy, HIV infection, or tuberculosis. Exome data of 4,327 East Asians (EAS) from the Exome Aggregation Consortium (ExAC) Browser (version ExAC.r0.3.1[33]) were retrieved as the general control population. Written informed consent conforming to the tenets of the Declaration of Helsinki was obtained from each participant before the study. The institutional review board of the Kunming Institute of Zoology approved this study.

NGS and Sanger Sequencing

Genomic DNA was extracted from whole blood with the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Scientific). Whole exome and coding regions of the targeted genes were captured with the NimbleGen SeqCap EZ Exome Kit v.3.0 and Choice Enrichment Kit (Roche NimbleGen), respectively, according to the manufacturer's protocols (NimbleGen SeqCap EZ Library SR User's Guide v.5.1). Genes identified in the WES discovery stage and/or located in previous GWAS and GWLS loci were subjected to the screening stage via targeted NGS. DNA probes for the coding regions of these target genes were designed and generated with the online NimbleDesign tool. All captured DNA libraries were sequenced with the Illumina HiSeq 4000/X ten Genome Analyzer (150-bp paired-end reads). Replication of potential susceptibility loci in the independent replication cohort (sample III) was performed by Sanger sequencing with the use of amplification and the sequencing primers in Table S1.

Genes within the Reported GWAS and GWLS Loci for Targeted NGS

We used the Phenotype-Genotype Integrator (PheGenI), which provides GWAS Catalog data,[34] to compile a complete gene list of all leprosy-associated GWAS loci. We also searched PubMed by using "leprosy," "linkage study," "association," "gene," and "genetic variants" as keywords. We obtained 30 genome-wide-significant loci from the available GWASs and GWLSs of leprosy.[7,8,13–19,35] Among these loci, 35 genes were captured by the targeted NGS (Table S2).

NGS Data Processing

Sequencing reads were trimmed and filtered with Trimmomatic.[36] The resulting reads were aligned to the human reference genome (UCSC Genome Browser hg19) with the Burrows-Wheeler Aligner.[37] The Genome Analysis Toolkit (GATK Best Practices)[38] was used for recalibrating base quality scores, realigning indels, and removing duplicates. We recalibrated variant scores by using the GATK VariantRecalibrator and ApplyRecalibration commands with the parameter "--ts_filter_level 99.0." All variants were annotated with ANNOVAR.[39]

Meta-analysis for *LACC1* rs3764147

We performed a meta-analysis to look for any association between *LACC1* rs3764147 and leprosy. We performed a literature search of PubMed to identify all available studies regarding the association between rs3764147 and leprosy up to May 2017. The keywords "*LACC1*," "*C13orf31*," "FAMIN," "chromosome 13 open reading

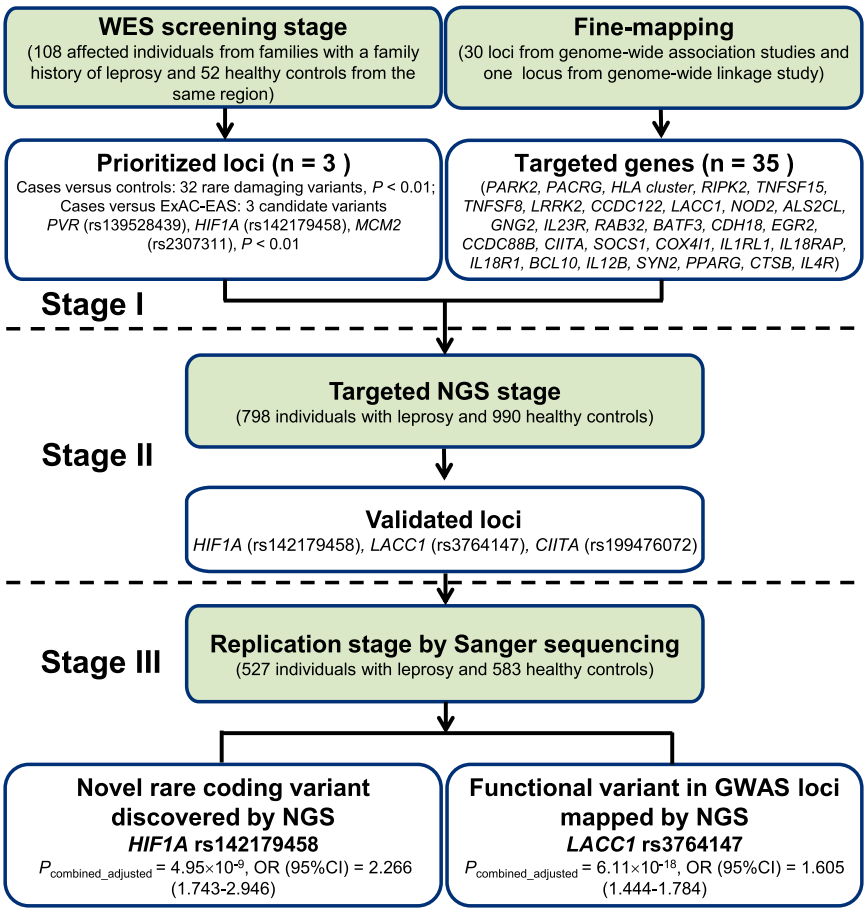

Figure 1. Strategy and Multiple Steps for Identifying Leprosy Susceptibility Genes in This Study

frame 31," "polymorphism," "SNP," and "leprosy" were used as the search terms. A total of 7,333 leprosy individuals and 10,329 control individuals from four previous studies[14,40–42] and this study were obtained for the analysis. The meta-analysis was performed by Review Manager (RevMan 5.2) according to the Cochran-Mantel-Haenszel method under a fixed effect. The heterogeneity was measured by the I^2 index.[43]

mRNA Expression Profiling

We performed a differential mRNA expression analysis to investigate the transcriptomic alterations of target genes during *M. leprae* infection by using the available data from the Gene Expression Omnibus (GEO). Three datasets were downloaded and reanalyzed: (1) dataset GEO: GSE100853, a genome-wide screen for expression quantitative trait loci before and after stimulation with *M. leprae* sonicate in whole blood cells from 51 unrelated individuals with borderline leprosy;[44] (2) dataset GEO: GSE95748, which includes the gene expression profiles of *M. leprae*-infected mouse Schwann cells at three time points (day 14, day 28, and pSLC [leprosy bacteria trigger the reprogramming of adult Schwann cells to progenitor/stem-like cells]) from an Affymetrix microarray;[45] and (3) dataset GEO: GSE74481, which includes the mRNA expression profiles of leprosy skin lesions from 24 individuals with MB (10 BB, 10 BL, and 4 LL), 20 individuals with PB (10 TT and 10 BT), 14 individuals with type I reaction (R1), and 10 individuals with type II reaction (R2), as well as normal skin biopsies from nine healthy individuals.[46]

Population Attributable Fraction of Top Leprosy Risk Loci

The population attributable fraction (PAF) is the proportional reduction of population disease burden that would occur if exposure to the risk allele were removed (e.g., if all alleles were wild-type).[47] In this study, we calculated the PAF for each top leprosy risk SNP (Table S3) to determine the effect of a risk factor on leprosy incidence in a population on the basis of odds ratio (OR) and allele frequency by using the equation

$$\mathrm{PAF} = \frac{P(\mathrm{OR} - 1)}{P(\mathrm{OR} - 1) + 1},$$

in which P is the frequency of the risk allele in the general population, and OR is the odds ratio of the risk allele in a previously reported GWAS and/or this study.

Statistical Analysis

Missense variants were rated as damaging when at least two of five prediction algorithms (SIFT,[48,49] PolyPhen-2 HumDiv, PolyPhen-2 HumVar,[50] LRT,[51] and MutationTaster[52]) suggested a potentially deleterious effect. Using Fisher's exact test for the single-site association analysis, we directly compared the allele frequencies of all of the damaging missense variants between individuals with leprosy and control individuals. We used logistic regression analysis with gender as the covariate to generate the adjusted p value ($p_{adjusted}$). We used all damaging variants with a minor allele frequency (MAF) < 1% (from the 1000 Genomes Project data[53]) for the gene-based burden test (command "--burden") to evaluate the enrichment of the rare functional alleles in individuals with leprosy by using the open-source C/C++ package PLINK/SEQ. The age of leprosy onset (for both MB and PB subtypes) was analyzed for association with *HIF1A* rs142179458 in individuals with MB and PB according to the Kaplan-Meier method, and the statistical significance was tested by the log-rank and Gehan-Breslow-Wilcoxon tests with GraphPad Prism v.5.01. The statistical power and sample size of the replication stages were estimated with Quanto software (v.1.2.4)[54] using the following parameters: MAF = 0.05, disease prevalence = 0.0001, and significance = 0.05 (two sided). We needed at least 257 pairs of case and control samples to capture an OR of 2.0 with a statistical power of 80% under an additive model. Sample sizes of the current replication stages were thus sufficient for validating the stage 1 association.

Results

Identifying Rare Missense Variants by WES in the Discovery Cohort

In the WES discovery stage, we obtained a total of 1,382.8 Gb of raw data for sample I (8.6 Gb per sample on average)

Figure 2. WES Identifies Risk Missense Variants in Individuals with Leprosy

(A) Presence of missense variants in 55 individuals with tuberculoid (TT) leprosy, 53 individuals with lepromatous (LL) leprosy, and 52 healthy control individuals. Shown are rare damaging variants with p values < 0.01. The top histogram shows the number of total rare damaging variants for each individual. The middle plot demonstrates gender information for each sample. The bottom plot on the right shows the presence or absence of each variant in the studied individuals. The bottom histogram on the left shows the significance of each variant (log[p] values). *HIF1A* c.1045G>A (p.Asp349Asn) is highlighted in a dashed box.

(B) Manhattan plot of the single-site association result of rare damaging variants in the discovery phase. A total of 17,917 rare damaging variants were used for making the plot, and 32 genes with rare damaging variants were identified with a cutoff p value < 0.01 (blue line). The three variants analyzed in the second stage validation analysis are marked in bold.

and achieved >100× coverage (64 million targeted exomes per sample). 239,653 genetic variants, including 61,664 coding variants (such as missense, nonsense, frameshift, and splicing sites), were identified after data quality filtering. Further *in silico* pathogenicity prediction analysis showed that 17,917 rare missense variants (MAF < 0.01 in the 1000 Genomes Project data[53]) could be categorized as damaging (defined by at least two of five prediction algorithms[48–52] suggesting a potentially deleterious effect). The summary statistics of the 17,917 rare damaging missense variants are shown in Table S4. We observed no significant SNPs when comparing individuals with LL against individuals with TT. The allele frequencies of 32 rare damaging variants were significantly different between individuals with leprosy and control individuals ($p < 0.01$; Figure 2; listed in Table S5). To cross-validate

Table 2. Association between Leprosy and *HIF1A* rs142179458 and *LACC1* rs3764147

Stage	Sample Size		Allele Count (Alternative/Reference)		P	$p_{adjusted}$[a]	OR	95% CI
	Leprosy Individuals	Control Individuals	Leprosy Individuals	Control Individuals				
***H1F1A* rs142179458: c.1045G>A (p.Asp349Asn)**								
WES (Wenshan)	108	52	25/191	2/102	2.34×10^{-3}	1.12×10^{-2}	6.675	1.550–28.749
Reference control (ExAC-EAS)	–	4,327	–	227/8,423	1.90×10^{-9}	–	4.857	3.137–7.520
Targeted NGS (Wenshan)	798	990	124/1,472	68/1,912	1.47×10^{-8}	1.08×10^{-7}	2.369	1.749–3.208
Replication (Yuxi)	527	583	21/1,033	18/1,148	4.25×10^{-1}	4.91×10^{-1}	1.297	0.687–2.447
Combined (Southwest China)	1,433	1,625	170/2,696	88/3,162	4.07×10^{-10}	4.95×10^{-9}	2.266	1.743–2.946
Combined (All individuals)	1,433	5,952	170/2,696	315/11,585	1.72×10^{-16}	–	2.319	1.916–2.808
Meta-analysis	1,433	1,625	–	–	1.20×10^{-9}	–	2.266	1.737–2.957
***LACC1* rs3764147: c.760A>G (p.Ile254Val)**								
WES (Wenshan)	108	52	88/128	41/63	0.918	0.593	1.056	0.655–1.704
Reference control (ExAC-EAS)	–	4,327	–	2,695/5,953	3.70×10^{-3}	–	1.519	1.153–2.000
Targeted NGS (Wenshan)	798	990	637/959	619/1,361	8.31×10^{-8}	1.33×10^{-7}	1.460	1.272–1.677
Replication (Yuxi)	527	583	447/607	319/847	1.01×10^{-13}	4.37×10^{-12}	1.955	1.637–2.335
Combined (Southwest China)	1,433	1,625	1,172/1,694	979/2,271	1.55×10^{-18}	6.11×10^{-18}	1.605	1.444–1.784
Meta-analysis	1,433	1,625	–	–	5.50×10^{-18}	–	1.596	1.435–1.775

Meta-analysis of the three-stage studies (WES, targeted NGS, and replication) was performed with the R package "metafor" (see Web Resources) by the Mantel-Haenszel method. Abbreviations are as follows: WES, samples were analyzed by whole-exome sequencing; targeted NGS, samples were analyzed by targeted next-generation sequencing technologies; replication, the Yuxi sample was used for replication; ExAC-EAS, East Asian population data from the ExAC Browser[33] was used as the general control population.[a]The p values were adjusted for gender.

the results, we used the dataset ExAC-EAS[33] as the general population control for comparison. We further validated 3 of the 32 variants: rs139528439 (c.676G>A [p.Glu226Lys]; p = 0.002, OR = 17.200) in *PVR* (MIM: 173850), rs142179458 (c.1045G>A [p.Asp349Asn]; p = 0.002, OR = 6.675) in *HIF1A*, and rs2307311 (c.1999G>A [p.Val667Met]; p = 0.005, OR = 0.076) in *MCM2* (MIM: 116945) (Table S5). We subjected these three variants to the second-stage analysis by targeted NGS.

Validation of Leprosy-Associated Missense Variants by Targeted NGS

We performed targeted NGS for *PVR*, *HIF1A*, and *MCM2* in sample II. To fine-map the potentially functional variants that would account for the reported genome-wide-significant signals in previous GWAS and GWLS loci (Figure 1), we also included 35 genes located in these hit regions for consideration (Table S2). We obtained an average sequencing depth > 150× coverage for each sample. A total of 1,676 SNPs (including 1,011 in protein-coding regions) were annotated, resulting in a significance threshold of $p < 2.98 \times 10^{-5}$ (0.05/1,676 after Bonferroni correction).

Among these variants, 92 SNPs (including 82 within the *HLA* cluster) showed significant associations with leprosy ($p < 2.98 \times 10^{-5}$; Table S6). The seeming enrichment of significant SNPs in the *HLA* cluster was consistent with previous reports of a positive GWAS signal in this region.[14,16,24] Excluding these significant SNPs in the *HLA* region (Figure S1), we found the other significant variants in *HIF1A*, *TNFSF15* (MIM: 604052), *LACC1*, *MCM2*, *CCDC88B* (MIM: 611205), *CIITA* (MIM: 600005), *CTSB* (MIM: 116810), and *IL18R1* (MIM: 604494). Among these variants, only three were missense variants; these included *HIF1A* rs142179458 (c.1045G>A [p.Asp349Asn]), which was identified in the discovery stage ($p = 1.47 \times 10^{-8}$, OR [95% CI] = 2.369 [1.749–3.208]; Figure S2). Two other missense variants in *LACC1* (rs3764147 [c.760A>G (p.Ile254Val)]; $p = 8.31 \times 10^{-8}$, OR [95% CI] = 1.460 [1.272–1.677]; Figure S3) and *CIITA* (rs199476072 [c.2356C>A (p.Gln786Lys)]; $p = 5.16 \times 10^{-6}$, OR [95% CI] = 22.57 [3.01–169.3]) were significantly associated with leprosy (Tables 2 and S6). The program-affiliated prediction for pathogenicity showed that these two variants were damaging, which might explain the significant GWAS signals for these two genes.[14,18] For the GWLS hit *PRKN-PACRG*,[7] we observed no functional SNPs significantly associated with leprosy in our deep sequencing of this

region, whereas one *PRKN* missense variant (rs1801582) showed suggestive significance ($p = 3.93 \times 10^{-4}$; Table S6 and Figure S4). Further studies will be needed to map the functional variants in this region.

Independent Replication of Associations between Leprosy and *HIF1A* rs142179458, *LACC1* rs3764147, and *CIITA* rs199476072

We replicated the associations between leprosy and rs142179458 (*HIF1A* c.1045G>A [p.Asp349Asn]), rs3764147 (*LACC1* c.760A>G [p.Ile254Val]), and rs199476072 (*CIITA* c.2356C>A [p.Gln786Lys]) by genotyping these three variants in an independent population from the Yuxi Prefecture (sample III). *HIF1A* rs142179458 had the same risk direction as in the first- and second-stage analyses, although the association did not reach statistical significance ($p_{adjusted} = 4.91 \times 10^{-1}$, OR [95% confidence interval (CI)] = 1.297 [0.687–2.447]; Table 2). However, we observed exome-wide significance for rs142179458 in the joint analysis ($p_{adjusted} = 4.95 \times 10^{-9}$, OR [95% CI] = 2.266 [1.743–2.946]) and meta-analysis ($p_{meta} = 1.20 \times 10^{-9}$, OR [95% CI] = 2.266 [1.737–2.957]) combining all Chinese samples from the three stages (1,433 affected individuals and 1,625 control individuals). When we combined the 4,327 ExAC-EAS[33] subjects with the control samples to achieve a large sample size for comparison, the association between *HIF1A* rs142179458 and leprosy was even more significant ($p = 1.72 \times 10^{-16}$). Of note, we found a striking association between rs142179458 and the MB subtype ($p_{adjusted} = 1.54 \times 10^{-8}$, OR [95% CI] = 2.346 [1.867–3.347]; Figure S5) and LL subtype ($p_{adjusted} = 2.42 \times 10^{-7}$, OR [95% CI] = 2.864 [1.838–4.087]), whereas the associations with the PB subtype ($p_{adjusted} = 4.95 \times 10^{-3}$; OR [95% CI] = 1.663 [1.422–2.745]) and TT subtype ($p_{adjusted} = 1.73 \times 10^{-2}$; OR [95% CI] = 1.690 [1.254–2.817]) were weak. These observations indicate that the significant association with leprosy might be caused by the skewing effect of the MB and/or LL individuals, as we have reported before.[22,23,30,55] This pattern supports the notion that genetic variants might be associated with leprosy polarization.[56] Further survival analysis showed that rs142179458-A is associated with earlier age of MB onset (Gehan-Breslow-Wilcoxon test p = 0.025; Figure S6).

The association between *LACC1* rs3764147 and leprosy was well validated in sample III ($p_{adjusted} = 4.37 \times 10^{-12}$, OR [95% CI] = 1.955 [1.637–2.335]; Table 2) and in the combined samples ($p_{adjusted} = 6.11 \times 10^{-18}$, OR [95% CI] = 1.605 [1.444–1.784]). The meta-analysis of *LACC1* rs3764147 in a total of 7,333 affected individuals and 10,329 control individuals confirmed the strong association between rs3764147 and leprosy ($p < 1.00 \times 10^{-5}$, OR [95% CI] = 1.55 [1.32–1.82]; Figure 3). The association between *LACC1* rs3764147 and leprosy was observed in both Chinese populations ($p < 1.00 \times 10^{-5}$, OR = 1.67) and non-Chinese populations ($p < 1.00 \times 10^{-5}$, OR = 1.47), suggesting that this gene contributes to leprosy in populations with different genetic backgrounds (Figure 3).

CIITA variant rs199476072 was extremely rare in sample III, and only one heterozygous carrier was observed in the leprosy group. We searched for this variant in other datasets, such as the ExAC dataset,[33] and found that rs199476072 had a MAF of 0.0001 (3/17,150) in the EAS population and was absent in other populations. A focused study with a larger sample size will be needed to explore the association between *CIITA* rs199476072 and leprosy.

In order to quantify the genetic contribution of a risk allele to leprosy, we compared the PAF of all established leprosy risk alleles (Table S3). *LACC1* rs3764147 showed the highest PAF among the missense variants, whereas *HIF1A* rs142179458 showed the highest PAF among the rare missense variants associated with leprosy (Figure 4).

Biological Involvement of *HIF1A*, *LACC1*, and *CIITA* in Leprosy

An evolutionary comparison of HIF1α p.Asp349Asn showed that residue Asp349 is highly conserved across different vertebrate species, including primates and rodentia (Figure S7). The mutant, rs142179458-A, has been reported to be associated with breast cancer and was predicted to decrease protein stability.[57] Therefore, rs142179458 is likely to be a potential loss-of-function variant, and mutant HIF1α might have decreased activity during physiological processes against infection. A gene-based burden test showed that individuals with leprosy have a higher burden of rare coding variants in *HIF1A* than healthy control individuals ($p = 1.00 \times 10^{-6}$), indicating more potentially disease-related missense variants in *HIF1A*. We evaluated the alterations in *HIF1A* mRNA expression during *M. leprae* infection on the basis of GEO: GSE100853.[44] With increasing dosages of *M. leprae* antigens (0, 5, and 20 μg/mL), the *HIF1A* mRNA expression level was significantly increased ($p < 1.00 \times 10^{-4}$; Figure 5A). We observed a similar expression pattern for the *HIF1A* mRNA level in dataset GEO: GSE95748;[45] along with the *M. leprae* infection in mouse Schwann cells, the *HIF1A* mRNA expression was increased (Figure 5B). Consistently, the *HIF1A* mRNA level in leprotic skin lesions from MB individuals was significantly higher than in controls individuals (p = 0.033, GEO: GSE7448;[46] Figure 5C). Moreover, mRNA expression of *HIF1A* was dramatically higher in the skin lesions of individuals with type I reaction (p = 0.011; Figure 5C) and type II reaction ($p < 1.00 \times 10^{-4}$; Figure 5C) than in healthy control individuals according to dataset GEO: GSE74481.[46]

Similar to *HIF1A* mRNA expression, *LACC1* mRNA expression was increased in response to *M. leprae* antigen treatment ($p < 1.00 \times 10^{-4}$; Figure 5D) according to GEO: GSE100853.[44] Unfortunately, no data were available for this gene in the other two datasets. The *CIITA* mRNA level was significantly higher in leprotic skin lesions of individuals with leprosy than in healthy control individuals ($p < 1.00 \times 10^{-4}$; Figure S8A) according to dataset GEO: GSE74481.[46] We also observed upregulated expression of

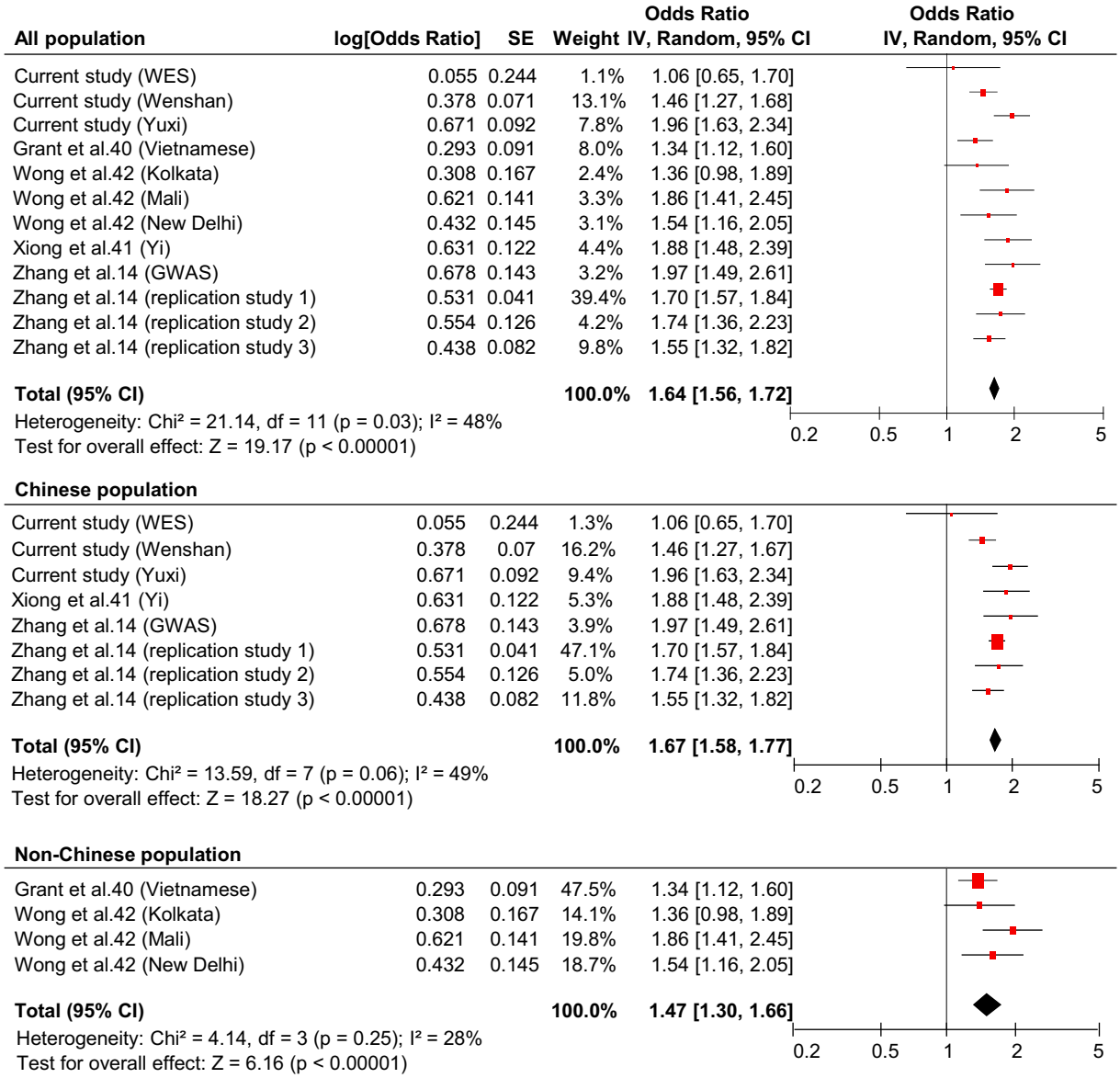

Figure 3. Meta-analyses of Association between *LACC1* rs3764147 and Leprosy in All Populations, Chinese Populations Only, and Non-Chinese Populations under the Allelic Model

The positions of the squares on the x axis indicate the effect size (OR) for each study, and the bars indicate the 95% confidence intervals (CIs) of the effect sizes. The effect size for each cohort was retrieved from the respective report[14,40–42] and this study. The forest plots were prepared by Review Manager (RevMan 5.2) according to the Mantel-Haenszel method with a fixed model. Abbreviations are as follows: I^2, heterogeneity (corresponding p values were measured by the Chi-square test); SE, standard error of the effect size (log [OR]); IV, inverse variance.

CIITA mRNA in whole blood cells after stimulation with a high concentration of *M. leprae* antigens (20 μg/mL; p = 0.004; Figure S8B) and in mouse Schwann cells at the early stage of *M. leprae* infection (14 day; p = 0.043) (Figure S8C). Together, the expression data indicate an active role for the three genes in leprosy.

Discussion

In recent years, we have seen a burst of large-scale analyses looking at the genetic basis of leprosy, and many susceptibility genes and variants have been identified.[9–11,14,16,58] In the present study, we used a three-stage analysis to identify potential protein-coding variants that contribute to leprosy susceptibility. In contrast to the GWAS-based analysis of common variants, we focused on rare and damaging variants in genes identified by WES and validated by targeted NGS. By first analyzing unrelated leprosy-affected individuals from two poles of the disease spectrum and unaffected subjects from high-risk families as matched healthy control individuals and then performing replication in a large sample, we were able to identify missense variant rs142179458 (c.1045G>A

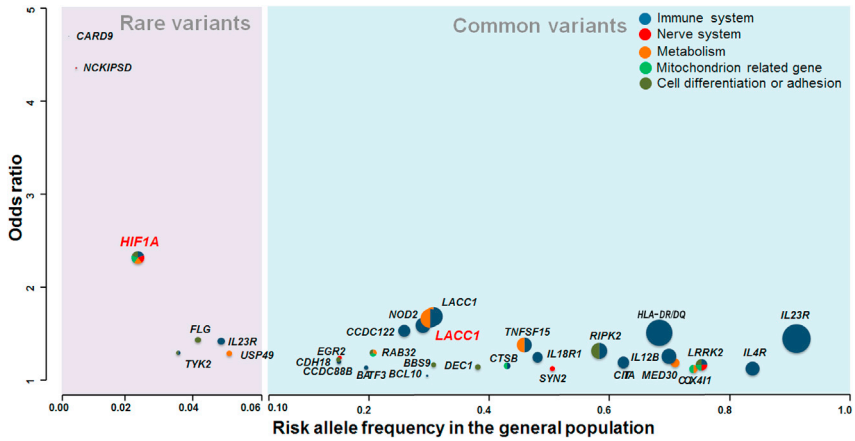

Figure 4. Rare and Common Variants Contributing to Leprosy Risk
The diameter of the circle is based on the PAF (Table S3), and different colors refer to potential roles of a gene in different pathways. *HIF1A* and *LACC1* in the current study are labeled in bold and red. The variant with a MAF < 0.05 in the CHB (Han Chinese in Beijing) 1000 Genomes population[53] is regarded as rare in this plot.

[p.Asp349Asn]) in *HIF1A* as being strongly associated with leprosy (Table 2), especially in MB individuals (Figure S5). The association was also present when we used the ExAC-EAS dataset[33] as the general control population (Table 2). By including additional genes located in the reported GWAS loci for targeted NGS, we validated the strong association between *LACC1* rs3764147 and leprosy (Table 2). These results support the theory that leprosy is genetically determined.[7,9–17,58]

The leprosy-associated variant rs142179458 (c.1045G>A [p.Asp349Asn]) is located in exon 8 of *HIF1A* in chromosomal region 14q23.2; its MAF is highest in East Asian populations and is much lower in other populations from the 1000 Genomes Project dataset.[53] In the ExAC Browser (accessed on January 30, 2018),[33] the largest exome database, we found a similar distribution pattern among populations for the leprosy risk allele rs142179458-A: 0.02719 in 9,432 East Asians, 0.00052 in 15,387 South Asians, 0.00020 in 17,201 Latinos, and 0.00037 in 12,017 Africans; this allele was absent in 76,156 Europeans and 5,071 Ashkenazi Jews. We speculated that *HIF1A* might have undergone natural selection, given that the evolutionary interaction between microbial pathogens (e.g., *M. leprae*) and humans was one of the main selection pressures that shaped genetic variations in human populations.[59] Indeed, *H1F1A* and its surrounding genes showed signals of selection, especially in Europeans and Africans, in the Haplotter database.[60] However, why this leprosy risk allele of *HIF1A* was specifically enriched in East Asian populations and how it contributed to the population risk to leprosy remain to be investigated. So far, one study has reported an association between rs142179458 and disease risk (breast cancer) in a Singaporean population.[57] *HIF1A* encodes the alpha subunit of hypoxia-inducible factor 1 (HIF1α), which is one of the major components of HIF-1. HIF-1 plays key roles in cellular and systemic oxygen homeostasis under hypoxia,[61] tumor progression,[62] and immune reactions.[61,63] HIF1α can be activated by environmental stimuli under normoxic conditions[64] and plays a role in inflammation[65] and chaperone-mediated autophagy.[66] *HIF1A* expression is induced by a number of cellular stresses, such as activation of the oxidative stress pathway under infection.[67,68] Increased oxidative stress has been observed in leprosy.[69,70] Dysregulation or dysfunction of HIF1A might disrupt this cellular signaling transduction in host responses to *M. leprae* infection. However, it remains to be investigated how *HIF1A* and other cellular-stress-related genes link the oxidative stress and hypoxia with *M. leprae* infection. In addition to immune-related genes, some autophagy-related genes, such as *IRGM* (MIM: 608212; rs13361189), have been reported to be associated with leprosy by affecting inflammatory cytokines.[71] In our previous study of missense variant c.7190T>C (p.Met2397Thr) (rs3761863) in *LRRK2*, we found that the leprosy protective residue Thr2397 could attenuate 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced autophagy in U251 cells.[31] The HIF1α-related signaling pathway is also involved in Parkinson disease (PD [MIM: 168600]).[72,73] These susceptibility genes shared between leprosy and PD, such as *HIF1A* in this study and the previously reported *LRRK2*, *PRKN*, and *PINK1*,[7,14,22,31,74] might suggest shared pathogenic pathways between leprosy and PD and reinforce the notion that leprosy is a neurological disease.[1–3]

The recent leprosy GWASs in Han Chinese have provided a valuable list of risk loci;[14–16,18,19] however, a limited number of genes have been replicated.[31,75] Moreover, despite the success of GWASs in identifying leprosy risk loci, it is difficult to understand the underlying pathological role and to interpret the biological function of these risk loci and genes in leprosy, especially when the risk loci reside in non-coding regions with limited annotations and unknown functions. After the first leprosy GWAS in Han Chinese, which identified *LACC1* variants to be associated with leprosy,[14] the association between these variants and leprosy has been replicated in Indians and Africans,[42] the Vietnamese,[40] and the Chinese Yi population.[41] By deep sequencing the coding region of *LACC1*, we found a strong association between the common missense variant rs3764147 and leprosy, indicating a potentially causative role of the mutant underlying the GWAS signal. Notably, *LACC1* rs3764147 showed the highest PAF among all leprosy risk missense variants (Figure 4), and *LACC1* affected the risk of a variety of immune diseases, such as inflammatory bowel disease (Crohn disease [MIM: 266600])[76,77] and systemic juvenile idiopathic arthritis

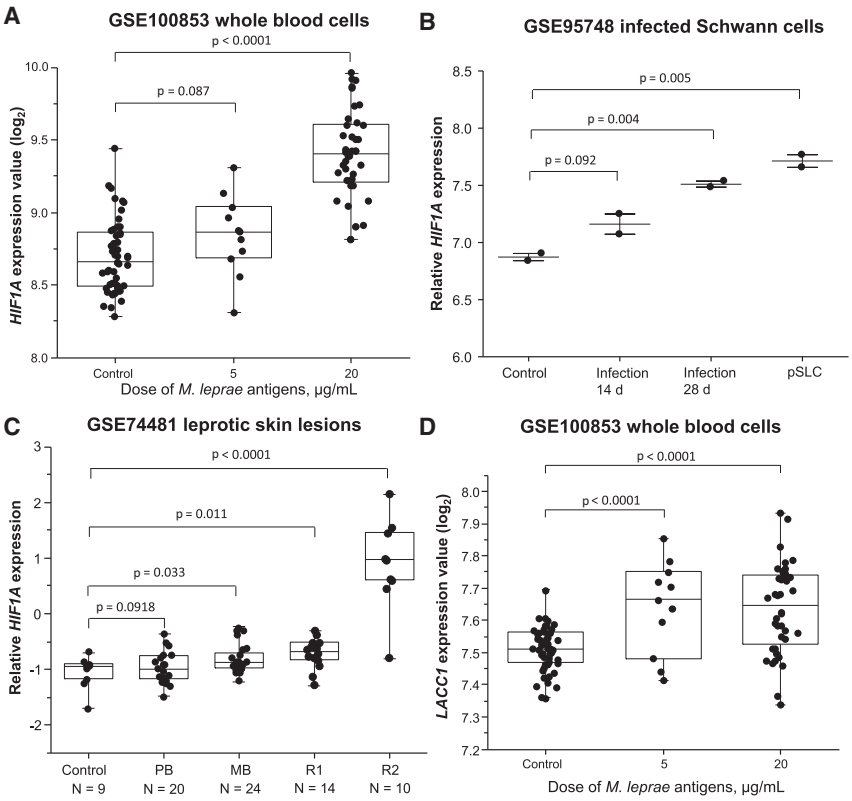

Figure 5. Upregulation of mRNA Expression Levels of *HIF1A* and *LACC1* in Leprotic Skin Lesions and Cells from Individuals with Leprosy Stimulated by *M. leprae* Sonicate

(A) Differential mRNA expression levels of *HIF1A* in whole blood cells of individuals with borderline leprosy. The expression dataset GEO: GSE100853,[44] which contains 51 unrelated Vietnamese individuals with borderline leprosy, was used for determining gene expression. Whole blood cells of each subject were stimulated with *M. leprae* sonicate (5 μg/mL for 11 individuals and 20 μg/mL for 40 individuals) or untreated (control).

(B) *HIF1A* mRNA expression in mouse Schwann cells infected with or without *M. leprae*. The analysis was based on expression dataset GEO: GSE95748.[45] The Schwann cells were harvested at successive stages of *M. leprae* infection for RNA extraction and hybridization with Affymetrix microarrays. Three time points of infection were used: days 14 and 28 after infection and pSLC (leprosy bacteria trigger the reprogramming of adult Schwann cells to progenitor/stem-like cells).[45]

(C) mRNA expression levels of *HIF1A* in leprotic skin lesions. The analysis was based on the microarray expression data GEO: GSE74481.[46] This dataset contains skin biopsies of 24 individuals with multibacillary (MB) leprosy, 20 individuals with paucibacillary (PB) leprosy, 14 individuals with type I reaction (R1), and 10 individuals with type II reaction (R2), as well as normal skin biopsies from 9 healthy individuals.

(D) Differential mRNA expression levels of *LACC1* in whole blood cells of individuals with borderline leprosy. Cells were stimulated with *M. leprae* sonicate, and gene expression is based on dataset GEO: GSE100853.[44]

The p values were determined with Student's t test. Error bars represent the standard error of the mean.

(MIM: 604302).[78] A recent functional study showed that *LACC1* is a central regulator of metabolic function and bioenergetic state of macrophages[79] and could increase innate receptor-induced responses.[80] We also observed positive associations in *TNFSF15*, *CCDC88B*, *CIITA*, *CTSB*, and *IL18R1* in our targeted sequencing stage (Table S6 and Figure S1), suggesting a list of targets for further validation.

CIITA is the upstream regulator of *HLA* genes.[81] Disruption of *CIITA* might lead to dysregulation of *HLA* genes, which are main hits for genetic susceptibility to leprosy.[14] In fact, we also found that *CIITA* mRNA is highly expressed in leprotic skin lesions of all types of leprosy (Figure S8). Although we could not validate the rare damaging variant of *CIITA* in an independent cohort because of sample size, its role in leprosy deserves further study.

During the preparation of our work, we noticed two recent publications about exome studies in Chinese individuals with leprosy.[58,82] Nine genes (*GAL3ST4* [MIM: 608235], *CHGB* [MIM: 118920], *NCKIPSD* [MIM: 606671], *CARD9* [MIM: 607212], *IL23R* [MIM: 607562], *FLG* [MIM: 135940], *USP49*, *SLC29A3* [MIM: 612373], and *IL27* [MIM: 608273]) were said to be associated with leprosy susceptibility in these studies.[58,82] We checked the risk variants in the above genes in our WES data (Table S7) but failed to find any association between these genes and leprosy in our samples. The main reasons for this might be the relatively small sample size of our initial WES discovery cohort and the extremely low frequency of the reported risk protein-coding variants (e.g., MAF = 0.0004 for *NCKIPSD* rs145562243 and MAF = 0.0006 for *CARD9* rs149308743[58]). Evidently, large sample sizes are needed for further validation of our current results and the recently reported ones.[58,82]

In summary, we have discovered missense variants contributing to leprosy risk through WES and targeted NGS in Chinese from Southwest China. We have provided genetic and expressional evidence to indicate *HIF1A* and *LACC1* as susceptibility genes for leprosy. Further studies are needed to validate the associations in more independent populations and to functionally characterize the roles of *HIF1A* and *LACC1* in the development of leprosy.

Supplemental Data

Supplemental Data include eight figures and seven tables and can be found with this article online at https://doi.org/10.1016/j.ajhg.2018.03.006.

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Web Resources

1000 Genomes Project, http://browser.1000genomes.org/index.html
dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/
ExAC Browser, http://exac.broadinstitute.org/
GATK Best Practices, http://www.broadinstitute.org/gatk/guide/topic?name=best-practices
Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/
Haplotter, http://haplotter.uchicago.edu/
NimbleDesign, https://design.nimblegen.com/nimbledesign/app/login?execution=e1s1
OMIM, http://www.omim.org/
PheGenI, http://www.ncbi.nlm.nih.gov/gap/phegeni
PLINK/SEQ, https://atgu.mgh.harvard.edu/plinkseq/
PubMed, https://www.ncbi.nlm.nih.gov/pubmed
R package "metafor," https://cran.r-project.org/web/packages/metafor/index.html
RevMan 5.2, http://tech.cochrane.org/revman
UCSC Genome Browser, https://genome.ucsc.edu/

References

1. Britton, W.J., and Lockwood, D.N. (2004). Leprosy. Lancet *363*, 1209–1219.
2. Madigan, C.A., Cambier, C.J., Kelly-Scumpia, K.M., Scumpia, P.O., Cheng, T.Y., Zailaa, J., Bloom, B.R., Moody, D.B., Smale, S.T., Sagasti, A., et al. (2017). A macrophage response to *Mycobacterium leprae* phenolic glycolipid initiates nerve damage in leprosy. Cell *170*, 973–985.e10.
3. Lancet Neurology (2009). Leprosy as a neurological disease. Lancet Neurol. *8*, 217.
4. World Health Organization (2015). Global leprosy update, 2015: Time for action, accountability and inclusion. Wkly. Epidemiol. Rec. *91*, 405–420.
5. Ridley, D.S., and Jopling, W.H. (1966). Classification of leprosy according to immunity. A five-group system. Int. J. Lepr. Other Mycobact. Dis. *34*, 255–273.
6. World Health Organization (1994). Chemotherapy of leprosy. Report of a WHO study group. World Health Organ. Tech. Rep. Ser. *847*, 1–24.
7. Mira, M.T., Alcaïs, A., Nguyen, V.T., Moraes, M.O., Di Flumeri, C., Vu, H.T., Mai, C.P., Nguyen, T.H., Nguyen, N.B., Pham, X.K., et al. (2004). Susceptibility to leprosy is associated with PARK2 and PACRG. Nature *427*, 636–640.
8. Mira, M.T., Alcaïs, A., Van Thuc, N., Thai, V.H., Huong, N.T., Ba, N.N., Verner, A., Hudson, T.J., Abel, L., and Schurr, E. (2003). Chromosome 6q25 is linked to susceptibility to leprosy in a Vietnamese population. Nat. Genet. *33*, 412–415.
9. Alter, A., Grant, A., Abel, L., Alcaïs, A., and Schurr, E. (2011). Leprosy as a genetic disease. Mamm. Genome *22*, 19–31.
10. Zhang, D.-F., Wang, D., Li, Y.-Y., and Yao, Y.-G. (2016). Integrative analyses of leprosy susceptibility genes indicate a common autoimmune profile. J. Dermatol. Sci. *82*, 18–27.
11. Misch, E.A., Berrington, W.R., Vary, J.C., Jr., and Hawn, T.R. (2010). Leprosy and the human genome. Microbiol. Mol. Biol. Rev. *74*, 589–620.
12. Alter, A., Alcaïs, A., Abel, L., and Schurr, E. (2008). Leprosy as a genetic model for susceptibility to common infectious diseases. Hum. Genet. *123*, 227–235.
13. Liu, H., Bao, F., Irwanto, A., Fu, X., Lu, N., Yu, G., Yu, Y., Sun, Y., Low, H., Li, Y., et al. (2013). An association study of TOLL and CARD with leprosy susceptibility in Chinese population. Hum. Mol. Genet. *22*, 4430–4437.
14. Zhang, F.R., Huang, W., Chen, S.M., Sun, L.D., Liu, H., Li, Y., Cui, Y., Yan, X.X., Yang, H.T., Yang, R.D., et al. (2009). Genomewide association study of leprosy. N. Engl. J. Med. *361*, 2609–2618.
15. Zhang, F., Liu, H., Chen, S., Low, H., Sun, L., Cui, Y., Chu, T., Li, Y., Fu, X., Yu, Y., et al. (2011). Identification of two new loci at IL23R and RAB32 that influence susceptibility to leprosy. Nat. Genet. *43*, 1247–1251.
16. Wang, Z., Sun, Y., Fu, X., Yu, G., Wang, C., Bao, F., Yue, Z., Li, J., Sun, L., Irwanto, A., et al. (2016). A large-scale genome-wide association and meta-analysis identified four novel susceptibility loci for leprosy. Nat. Commun. *7*, 13760.
17. Fava, V.M., Manry, J., Cobat, A., Orlova, M., Van Thuc, N., Moraes, M.O., Sales-Marques, C., Stefani, M.M., Latini, A.C., Belone, A.F., et al. (2017). A genome wide association study identifies a lncRna as risk factor for pathological inflammatory responses in leprosy. PLoS Genet. *13*, e1006637.
18. Liu, H., Irwanto, A., Fu, X., Yu, G., Yu, Y., Sun, Y., Wang, C., Wang, Z., Okada, Y., Low, H., et al. (2015). Discovery of six new susceptibility loci and analysis of pleiotropic effects in leprosy. Nat. Genet. *47*, 267–271.
19. Liu, H., Irwanto, A., Tian, H., Fu, X., Yu, Y., Yu, G., Low, H., Chu, T., Li, Y., Shi, B., et al. (2012). Identification of IL18RAP/IL18R1 and IL12B as leprosy risk genes demonstrates shared pathogenesis between inflammation and infectious diseases. Am. J. Hum. Genet. *91*, 935–941.
20. Mazini, P.S., Alves, H.V., Reis, P.G., Lopes, A.P., Sell, A.M., Santos-Rosa, M., Visentainer, J.E., and Rodrigues-Santos, P. (2016). Gene association with leprosy: A review of published data. Front. Immunol. *6*, 658.
21. Modlin, R.L. (2010). The innate immune response in leprosy. Curr. Opin. Immunol. *22*, 48–54.
22. Wang, D., Zhang, D.-F., Feng, J.-Q., Li, G.-D., Li, X.-A., Yu, X.-F., Long, H., Li, Y.-Y., and Yao, Y.-G. (2016). Common variants in the PARL and PINK1 genes increase the risk to leprosy in Han Chinese from South China. Sci. Rep. *6*, 37086.
23. Wang, D., Zhang, D.F., Li, G.D., Bi, R., Fan, Y., Wu, Y., Yu, X.F., Long, H., Li, Y.Y., and Yao, Y.G. (2017). A pleiotropic effect of the APOE gene: association of APOE polymorphisms with multibacillary leprosy in Han Chinese from Southwest China. Br. J. Dermatol. https://doi.org/10.1111/bjd.16020.
24. Zhang, F.R., Liu, H., Irwanto, A., Fu, X.A., Li, Y., Yu, G.Q., Yu, Y.X., Chen, M.F., Low, H.Q., Li, J.H., et al. (2013). HLA-B*13:01 and the dapsone hypersensitivity syndrome. N. Engl. J. Med. *369*, 1620–1628.
25. Manolio, T.A., Collins, F.S., Cox, N.J., Goldstein, D.B., Hindorff, L.A., Hunter, D.J., McCarthy, M.I., Ramos, E.M., Cardon,

L.R., Chakravarti, A., et al. (2009). Finding the missing heritability of complex diseases. Nature *461*, 747–753.
26. McClellan, J., and King, M.C. (2010). Genetic heterogeneity in human disease. Cell *141*, 210–217.
27. Asimit, J., and Zeggini, E. (2010). Rare variant association analysis methods for complex traits. Annu. Rev. Genet. *44*, 293–308.
28. Timpson, N.J., Greenwood, C.M.T., Soranzo, N., Lawson, D.J., and Richards, J.B. (2018). Genetic architecture: the shape of the genetic contribution to human traits and disease. Nat. Rev. Genet. *19*, 110–124.
29. Wang, D., Feng, J.-Q., Li, Y.-Y., Zhang, D.-F., Li, X.-A., Li, Q.-W., and Yao, Y.-G. (2012). Genetic variants of the MRC1 gene and the IFNG gene are associated with leprosy in Han Chinese from Southwest China. Hum. Genet. *131*, 1251–1260.
30. Wang, D., Li, G.-D., Fan, Y., Zhang, D.-F., Bi, R., Yu, X.-F., Long, H., Li, Y.-Y., and Yao, Y.-G. (2017). The mtDNA replication-related genes TFAM and POLG are associated with leprosy in Han Chinese from Southwest China. J. Dermatol. Sci. *88*, 349–356.
31. Wang, D., Xu, L., Lv, L., Su, L.-Y., Fan, Y., Zhang, D.-F., Bi, R., Yu, D., Zhang, W., Li, X.-A., et al. (2015). Association of the LRRK2 genetic polymorphisms with leprosy in Han Chinese from Southwest China. Genes Immun. *16*, 112–119.
32. Li, Y.Y., Li, X.A., He, L., Wang, D., Chen, W.Y., Chen, L., Lu, J.B., and 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.
33. Lek, M., Karczewski, K.J., Minikel, E.V., Samocha, K.E., Banks, E., Fennell, T., O'Donnell-Luria, A.H., Ware, J.S., Hill, A.J., Cummings, B.B., et al.; Exome Aggregation Consortium (2016). Analysis of protein-coding genetic variation in 60,706 humans. Nature *536*, 285–291.
34. MacArthur, J., Bowler, E., Cerezo, M., Gil, L., Hall, P., Hastings, E., Junkins, H., McMahon, A., Milano, A., Morales, J., et al. (2017). The new NHGRI-EBI Catalog of published genome-wide association studies (GWAS Catalog). Nucleic Acids Res. *45* (D1), D896–D901.
35. Siddiqui, M.R., Meisner, S., Tosh, K., Balakrishnan, K., Ghei, S., Fisher, S.E., Golding, M., Shanker Narayan, N.P., Sitaraman, T., Sengupta, U., et al. (2001). A major susceptibility locus for leprosy in India maps to chromosome 10p13. Nat. Genet. *27*, 439–441.
36. Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics *30*, 2114–2120.
37. Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics *25*, 1754–1760.
38. DePristo, M.A., Banks, E., Poplin, R., Garimella, K.V., Maguire, J.R., Hartl, C., Philippakis, A.A., del Angel, G., Rivas, M.A., Hanna, M., et al. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat. Genet. *43*, 491–498.
39. Wang, K., Li, M., and Hakonarson, H. (2010). ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. *38*, e164.
40. Grant, A.V., Alter, A., Huong, N.T., Orlova, M., Van Thuc, N., Ba, N.N., Thai, V.H., Abel, L., Schurr, E., and Alcais, A. (2012). Crohn's disease susceptibility genes are associated with leprosy in the Vietnamese population. J. Infect. Dis. *206*, 1763–1767.
41. Xiong, J.H., Mao, C., Sha, X.W., Jin, Z., Wang, H., Liu, Y.Y., and Ning, Y. (2016). Association between genetic variants in NOD2, C13orf31, and CCDC122 genes and leprosy among the Chinese Yi population. Int. J. Dermatol. *55*, 65–69.
42. Wong, S.H., Hill, A.V., Vannberg, F.O.; and India-Africa-United Kingdom Leprosy Genetics Consortium (2010). Genomewide association study of leprosy. N. Engl. J. Med. *362*, 1446–1447, author reply 1447–1448.
43. Higgins, J.P., and Thompson, S.G. (2002). Quantifying heterogeneity in a meta-analysis. Stat. Med. *21*, 1539–1558.
44. Manry, J., Nédélec, Y., Fava, V.M., Cobat, A., Orlova, M., Thuc, N.V., Thai, V.H., Laval, G., Barreiro, L.B., and Schurr, E. (2017). Deciphering the genetic control of gene expression following *Mycobacterium leprae* antigen stimulation. PLoS Genet. *13*, e1006952.
45. Masaki, T., Qu, J., Cholewa-Waclaw, J., Burr, K., Raaum, R., and Rambukkana, A. (2013). Reprogramming adult Schwann cells to stem cell-like cells by leprosy bacilli promotes dissemination of infection. Cell *152*, 51–67.
46. Belone, Ade.F., Rosa, P.S., Trombone, A.P., Fachin, L.R., Guidella, C.C., Ura, S., Barreto, J.A., Pinilla, M.G., de Carvalho, A.F., Carraro, D.M., et al. (2015). Genome-wide screening of mRNA expression in leprosy patients. Front. Genet. *6*, 334.
47. Rockhill, B., Newman, B., and Weinberg, C. (1998). Use and misuse of population attributable fractions. Am. J. Public Health *88*, 15–19.
48. Ng, P.C., and Henikoff, S. (2003). SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res. *31*, 3812–3814.
49. Kumar, P., Henikoff, S., and Ng, P.C. (2009). Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat. Protoc. *4*, 1073–1081.
50. Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S., and Sunyaev, S.R. (2010). A method and server for predicting damaging missense mutations. Nat. Methods *7*, 248–249.
51. Chun, S., and Fay, J.C. (2009). Identification of deleterious mutations within three human genomes. Genome Res. *19*, 1553–1561.
52. Schwarz, J.M., Cooper, D.N., Schuelke, M., and Seelow, D. (2014). MutationTaster2: Mutation prediction for the deep-sequencing age. Nat. Methods *11*, 361–362.
53. Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Kang, H.M., Korbel, J.O., Marchini, J.L., McCarthy, S., McVean, G.A., Abecasis, G.R.; and 1000 Genomes Project Consortium (2015). A global reference for human genetic variation. Nature *526*, 68–74.
54. Gauderman, W.J. (2002). Sample size requirements for matched case-control studies of gene-environment interaction. Stat. Med. *21*, 35–50.
55. Xiang, Y.-L., Zhang, D.-F., Wang, D., Li, Y.-Y., and Yao, Y.-G. (2015). Common variants of OPA1 conferring genetic susceptibility to leprosy in Han Chinese from Southwest China. J. Dermatol. Sci. *80*, 133–141.
56. Gaschignard, J., Grant, A.V., Thuc, N.V., Orlova, M., Cobat, A., Huong, N.T., Ba, N.N., Thai, V.H., Abel, L., Schurr, E., and Alcaïs, A. (2016). Pauci- and multibacillary leprosy: Two distinct, genetically neglected diseases. PLoS Negl. Trop. Dis. *10*, e0004345.
57. Chan, C.H.T., Munusamy, P., Loke, S.Y., Koh, G.L., Wong, E.S.Y., Law, H.Y., Yoon, C.S., Tan, M.H., Yap, Y.S., Ang, P., and Lee, A.S.G. (2017). Identification of novel breast cancer risk loci. Cancer Res. *77*, 5428–5437.

58. Liu, H., Wang, Z., Li, Y., Yu, G., Fu, X., Wang, C., Liu, W., Yu, Y., Bao, F., Irwanto, A., et al. (2017). Genome-wide analysis of protein-coding variants in leprosy. J. Invest. Dermatol. *137*, 2544–2551.
59. Karlsson, E.K., Kwiatkowski, D.P., and Sabeti, P.C. (2014). Natural selection and infectious disease in human populations. Nat. Rev. Genet. *15*, 379–393.
60. Voight, B.F., Kudaravalli, S., Wen, X., and Pritchard, J.K. (2006). A map of recent positive selection in the human genome. PLoS Biol. *4*, e72.
61. Hellwig-Bürgel, T., Stiehl, D.P., Wagner, A.E., Metzen, E., and Jelkmann, W. (2005). Review: Hypoxia-inducible factor-1 (HIF-1): A novel transcription factor in immune reactions. J. Interferon Cytokine Res. *25*, 297–310.
62. Ajduković, J. (2016). HIF-1–A big chapter in the cancer tale. Exp. Oncol. *38*, 9–12.
63. Metheni, M., Lombès, A., Bouillaud, F., Batteux, F., and Langsley, G. (2015). HIF-1α induction, proliferation and glycolysis of Theileria-infected leukocytes. Cell. Microbiol. *17*, 467–472.
64. Majmundar, A.J., Wong, W.J., and Simon, M.C. (2010). Hypoxia-inducible factors and the response to hypoxic stress. Mol. Cell *40*, 294–309.
65. Mladenova, D.N., Dahlstrom, J.E., Tran, P.N., Benthani, F., Bean, E.G., Ng, I., Pangon, L., Currey, N., and Kohonen-Corish, M.R. (2015). HIF1α deficiency reduces inflammation in a mouse model of proximal colon cancer. Dis. Model. Mech. *8*, 1093–1103.
66. Ferreira, J.V., Fôfo, H., Bejarano, E., Bento, C.F., Ramalho, J.S., Girão, H., and Pereira, P. (2013). STUB1/CHIP is required for HIF1A degradation by chaperone-mediated autophagy. Autophagy *9*, 1349–1366.
67. Deshmane, S.L., Mukerjee, R., Fan, S., Del Valle, L., Michiels, C., Sweet, T., Rom, I., Khalili, K., Rappaport, J., Amini, S., and Sawaya, B.E. (2009). Activation of the oxidative stress pathway by HIV-1 Vpr leads to induction of hypoxia-inducible factor 1alpha expression. J. Biol. Chem. *284*, 11364–11373.
68. Movafagh, S., Crook, S., and Vo, K. (2015). Regulation of hypoxia-inducible factor-1a by reactive oxygen species: New developments in an old debate. J. Cell. Biochem. *116*, 696–703.
69. Jyothi, P., Riyaz, N., Nandakumar, G., and Binitha, M.P. (2008). A study of oxidative stress in paucibacillary and multibacillary leprosy. Indian J. Dermatol. Venereol. Leprol. *74*, 80.
70. Schalcher, T.R., Borges, R.S., Coleman, M.D., Batista Júnior, J., Salgado, C.G., Vieira, J.L., Romão, P.R., Oliveira, F.R., and Monteiro, M.C. (2014). Clinical oxidative stress during leprosy multidrug therapy: Impact of dapsone oxidation. PLoS ONE *9*, e85712.
71. Yang, D., Chen, J., Shi, C., Jing, Z., and Song, N. (2014). Autophagy gene polymorphism is associated with susceptibility to leprosy by affecting inflammatory cytokines. Inflammation *37*, 593–598.
72. Rajagopalan, S., Rane, A., Chinta, S.J., and Andersen, J.K. (2016). Regulation of ATP13A2 via PHD2-HIF1α signaling is critical for cellular iron homeostasis: Implications for Parkinson's disease. J. Neurosci. *36*, 1086–1095.
73. Dong, S.Y., Guo, Y.J., Feng, Y., Cui, X.X., Kuo, S.H., Liu, T., and Wu, Y.C. (2016). The epigenetic regulation of HIF-1α by SIRT1 in MPP(+) treated SH-SY5Y cells. Biochem. Biophys. Res. Commun. *470*, 453–459.
74. Nuytemans, K., Theuns, J., Cruts, M., and Van Broeckhoven, C. (2010). Genetic etiology of Parkinson disease associated with mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 genes: A mutation update. Hum. Mutat. *31*, 763–780.
75. Sales-Marques, C., Salomão, H., Fava, V.M., Alvarado-Arnez, L.E., Amaral, E.P., Cardoso, C.C., Dias-Batista, I.M., da Silva, W.L., Medeiros, P., da Cunha Lopes Virmond, M., et al. (2014). NOD2 and CCDC122-LACC1 genes are associated with leprosy susceptibility in Brazilians. Hum. Genet. *133*, 1525–1532.
76. Jostins, L., Ripke, S., Weersma, R.K., Duerr, R.H., McGovern, D.P., Hui, K.Y., Lee, J.C., Schumm, L.P., Sharma, Y., Anderson, C.A., et al.; International IBD Genetics Consortium (IIBDGC) (2012). Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature *491*, 119–124.
77. Patel, N., El Mouzan, M.I., Al-Mayouf, S.M., Adly, N., Mohamed, J.Y., Al Mofarreh, M.A., Ibrahim, N., Xiong, Y., Zhao, Q., Al-Saleem, K.A., and Alkuraya, F.S. (2014). Study of Mendelian forms of Crohn's disease in Saudi Arabia reveals novel risk loci and alleles. Gut *63*, 1831–1832.
78. Wakil, S.M., Monies, D.M., Abouelhoda, M., Al-Tassan, N., Al-Dusery, H., Naim, E.A., Al-Younes, B., Shinwari, J., Al-Mohanna, F.A., Meyer, B.F., and Al-Mayouf, S. (2015). Association of a mutation in LACC1 with a monogenic form of systemic juvenile idiopathic arthritis. Arthritis Rheumatol. *67*, 288–295.
79. Cader, M.Z., Boroviak, K., Zhang, Q., Assadi, G., Kempster, S.L., Sewell, G.W., Saveljeva, S., Ashcroft, J.W., Clare, S., Mukhopadhyay, S., et al. (2016). C13orf31 (FAMIN) is a central regulator of immunometabolic function. Nat. Immunol. *17*, 1046–1056.
80. Lahiri, A., Hedl, M., Yan, J., and Abraham, C. (2017). Human LACC1 increases innate receptor-induced responses and a LACC1 disease-risk variant modulates these outcomes. Nat. Commun. *8*, 15614.
81. Steimle, V., Siegrist, C.A., Mottet, A., Lisowska-Grospierre, B., and Mach, B. (1994). Regulation of MHC class II expression by interferon-gamma mediated by the transactivator gene CIITA. Science *265*, 106–109.
82. Yuan, Y., You, Y., Wen, Y., Liu, J., Li, H., Zhang, Y., Wu, N., Liu, S., Zhang, S., Chen, J., et al. (2017). Identification of novel genetic loci GAL3ST4 and CHGB involved in susceptibility to leprosy. Sci. Rep. *7*, 16352.