

# 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.<sup>1–3</sup> 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).<sup>4</sup> Leprosy manifests in five clinical forms, including tuberculoid (TT), borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL), and lepromatous (LL).<sup>5</sup> For treatment purposes, the WHO has categorized leprosy as multibacillary (MB, including LL, BL, and BB) or paucibacillary (PB, including TT and BT).<sup>6</sup>

Previous genetic studies using a family-based association analysis,<sup>7,8</sup> candidate-gene strategy,<sup>9–13</sup> or genome-wide association study (GWAS)<sup>14–19</sup> 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.<sup>10,11,20–23</sup> The large-scale GWAS analysis of Chinese populations in the last decade greatly broadened our

knowledge regarding the genetic susceptibility to leprosy<sup>14–16,18,19</sup> and hypersensitivity to dapsone treatment.<sup>24</sup> 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.<sup>16</sup> Additional genes and variants with stronger effect sizes need to be identified to address the so-called “missing heritability.”<sup>25,26</sup> 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.<sup>27,28</sup>

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 <sup>a</sup>	Targeted NGS <sup>b</sup>	Replication
<b>Individuals with Leprosy</b>			
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%)
<b>Control Individuals</b>			
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.<sup>a</sup>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.

<sup>b</sup>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.<sup>22,23,29–31</sup> 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.<sup>32</sup> 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<sup>33</sup>) 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

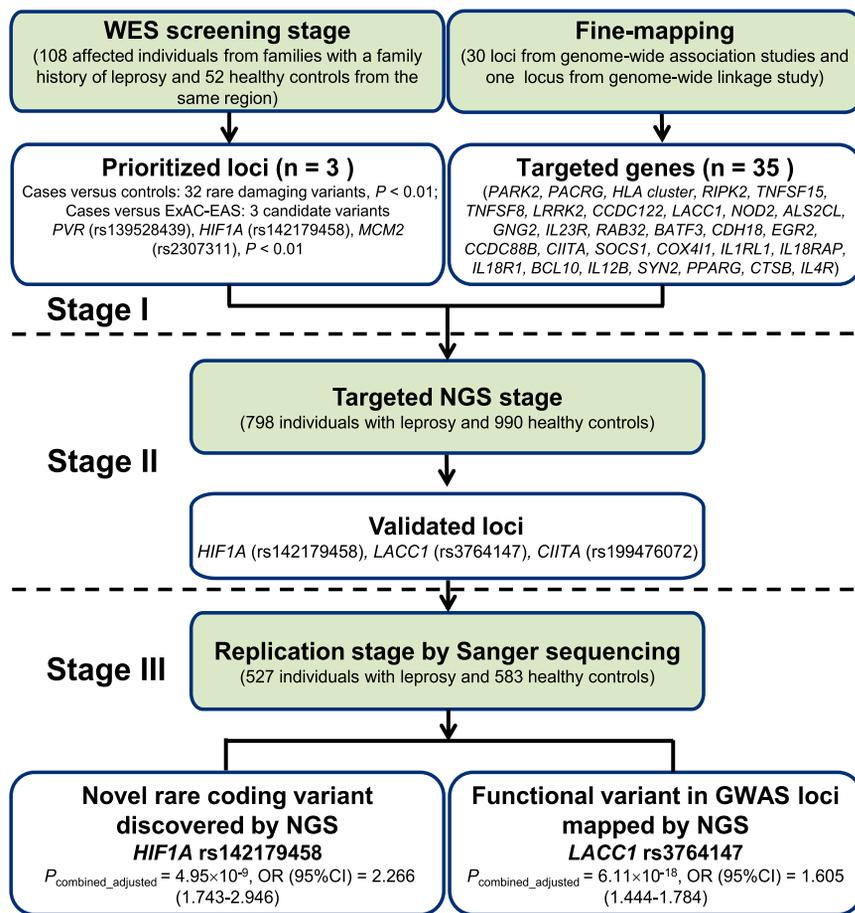
We used the Phenotype-Genotype Integrator (PheGenI), which provides GWAS Catalog data,<sup>34</sup> to compile a complete gene list of all leprosy-associated GWAS loci. We also searched PubMed by using “leprosy,” “linkage study,” “association,” “gene,” and “genetic variants” as keywords. We obtained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

### NGS Data Processing

Sequencing reads were trimmed and filtered with Trimmomatic.<sup>36</sup> The resulting reads were aligned to the human reference genome (UCSC Genome Browser hg19) with the Burrows-Wheeler Aligner.<sup>37</sup> The Genome Analysis Toolkit (GATK Best Practices)<sup>38</sup> 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.<sup>39</sup>

### 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**

### 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).<sup>47</sup> 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

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

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,<sup>48,49</sup> PolyPhen-2 HumDiv, PolyPhen-2 HumVar,<sup>50</sup> LRT,<sup>51</sup> and MutationTaster<sup>52</sup>) 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 ( $p_{\text{adjusted}}$ ). We used all damaging variants with a minor allele frequency (MAF)  $< 1\%$  (from the 1000 Genomes Project data<sup>53</sup>) 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)<sup>54</sup> 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.

### mRNA Expression Profiling

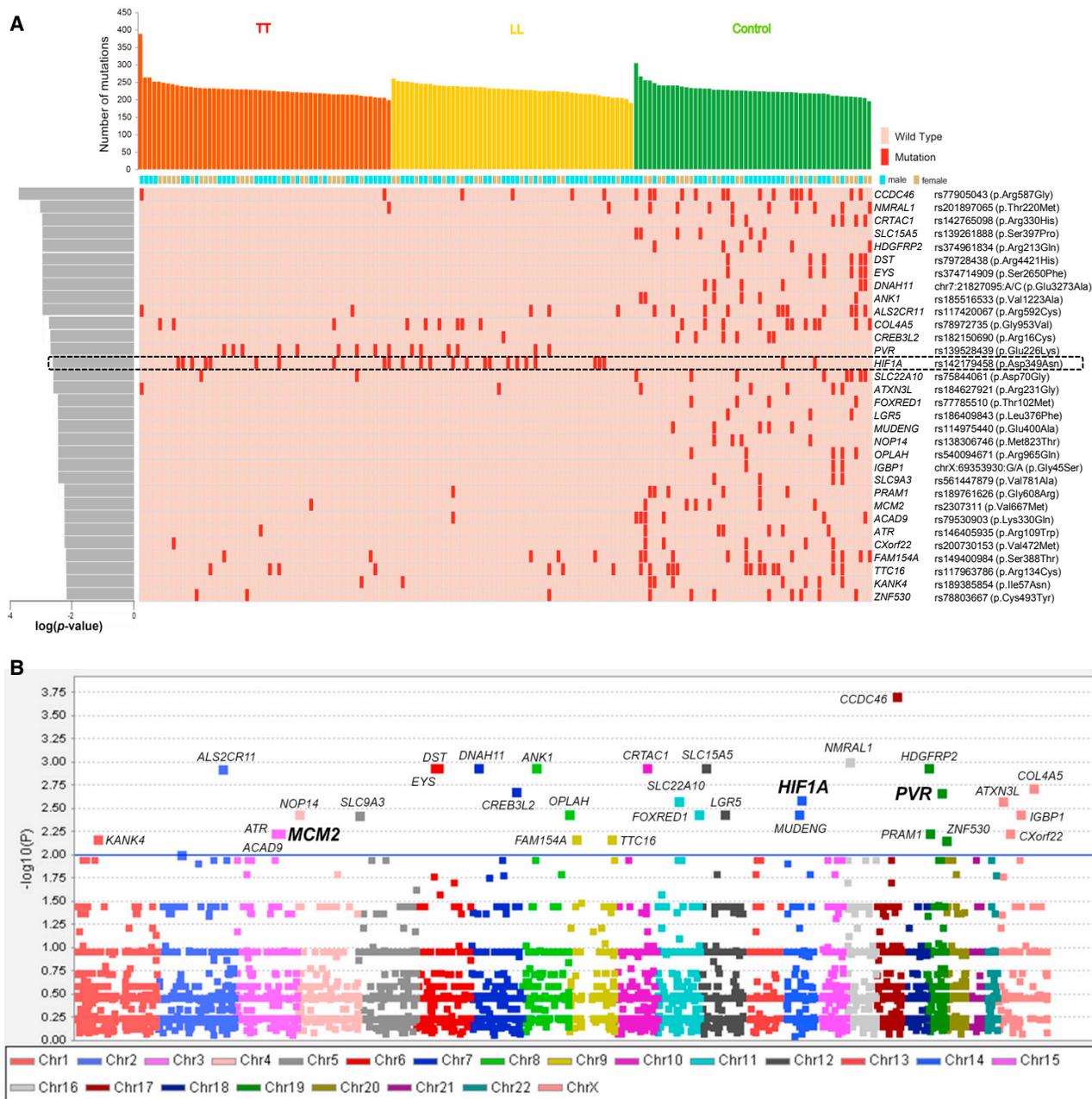
We performed a differential mRNA expression analysis to investigate the transcriptomic alterations of target genes during *M. leprae* infection by using the available data from the Gene Expression Omnibus (GEO). Three datasets were downloaded and reanalyzed: (1) dataset GEO: GSE100853, a genome-wide 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;<sup>44</sup> (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;<sup>45</sup> 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.<sup>46</sup>

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 ( $p_{\text{adjusted}}$ ). We used all damaging variants with a minor allele frequency (MAF)  $< 1\%$  (from the 1000 Genomes Project data<sup>53</sup>) 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)<sup>54</sup> 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 1 (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  $>100\times$  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<sup>53</sup>) could be categorized as damaging (defined by at least two of five prediction algo-

ri thms<sup>48–52</sup> 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 <sub>adjusted</sub> <sup>a</sup>	OR	95% CI
	Leprosy Individuals	Control Individuals	Leprosy Individuals	Control Individuals				
<b><i>HIF1A</i> rs142179458: c.1045G&gt;A (p.Asp349Asn)</b>								
WES (Wenshan)	108	52	25/191	2/102	2.34 × 10 <sup>-3</sup>	1.12 × 10 <sup>-2</sup>	6.675	1.550–28.749
Reference control (ExAC-EAS)	–	4,327	–	227/8,423	1.90 × 10 <sup>-9</sup>	–	4.857	3.137–7.520
Targeted NGS (Wenshan)	798	990	124/1,472	68/1,912	1.47 × 10 <sup>-8</sup>	1.08 × 10 <sup>-7</sup>	2.369	1.749–3.208
Replication (Yuxi)	527	583	21/1,033	18/1,148	4.25 × 10 <sup>-1</sup>	4.91 × 10 <sup>-1</sup>	1.297	0.687–2.447
Combined (Southwest China)	1,433	1,625	170/2,696	88/3,162	4.07 × 10 <sup>-10</sup>	4.95 × 10 <sup>-9</sup>	2.266	1.743–2.946
Combined (All individuals)	1,433	5,952	170/2,696	315/11,585	1.72 × 10 <sup>-16</sup>	–	2.319	1.916–2.808
Meta-analysis	1,433	1,625	–	–	1.20 × 10 <sup>-9</sup>	–	2.266	1.737–2.957
<b><i>LACC1</i> rs3764147: c.760A&gt;G (p.Ile254Val)</b>								
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 <sup>-3</sup>	–	1.519	1.153–2.000
Targeted NGS (Wenshan)	798	990	637/959	619/1,361	8.31 × 10 <sup>-8</sup>	1.33 × 10 <sup>-7</sup>	1.460	1.272–1.677
Replication (Yuxi)	527	583	447/607	319/847	1.01 × 10 <sup>-13</sup>	4.37 × 10 <sup>-12</sup>	1.955	1.637–2.335
Combined (Southwest China)	1,433	1,625	1,172/1,694	979/2,271	1.55 × 10 <sup>-18</sup>	6.11 × 10 <sup>-18</sup>	1.605	1.444–1.784
Meta-analysis	1,433	1,625	–	–	5.50 × 10 <sup>-18</sup>	–	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<sup>33</sup> was used as the general control population.<sup>a</sup>The p values were adjusted for gender.

the results, we used the dataset ExAC-EAS<sup>33</sup> 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.<sup>14,16,24</sup> 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.<sup>14,18</sup> For the GWLS hit *PRKN-PACRG*,<sup>7</sup> 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 ( $p_{\text{adjusted}} = 4.91 \times 10^{-1}$ , 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 ( $p_{\text{adjusted}} = 4.95 \times 10^{-9}$ , OR [95% CI] = 2.266 [1.743–2.946]) and meta-analysis ( $p_{\text{meta}} = 1.20 \times 10^{-9}$ , 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<sup>33</sup> 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 ( $p_{\text{adjusted}} = 1.54 \times 10^{-8}$ , OR [95% CI] = 2.346 [1.867–3.347]; Figure S5) and LL subtype ( $p_{\text{adjusted}} = 2.42 \times 10^{-7}$ , OR [95% CI] = 2.864 [1.838–4.087]), whereas the associations with the PB subtype ( $p_{\text{adjusted}} = 4.95 \times 10^{-3}$ ; OR [95% CI] = 1.663 [1.422–2.745]) and TT subtype ( $p_{\text{adjusted}} = 1.73 \times 10^{-2}$ ; 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.<sup>22,23,30,55</sup> This pattern supports the notion that genetic variants might be associated with leprosy polarization.<sup>56</sup> 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 ( $p_{\text{adjusted}} = 4.37 \times 10^{-12}$ , OR [95% CI] = 1.955 [1.637–2.335]; Table 2) and in the combined samples ( $p_{\text{adjusted}} = 6.11 \times 10^{-18}$ , 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).

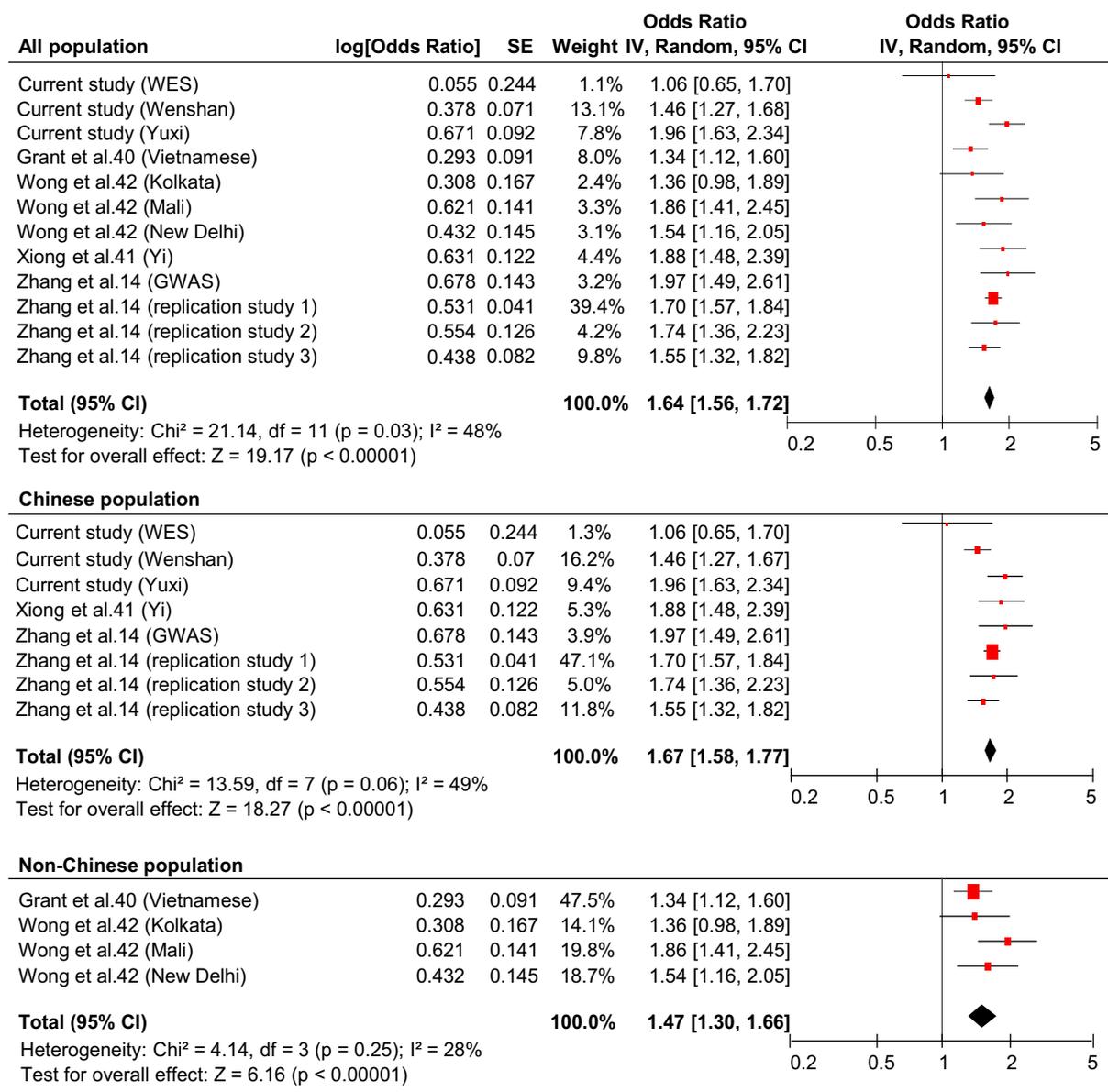
*CIITA* variant rs199476072 was extremely rare in sample III, and only one heterozygous carrier was observed in the leprosy group. We searched for this variant in other datasets, such as the ExAC dataset,<sup>33</sup> and found that rs199476072 had a MAF of 0.0001 (3/17,150) in the EAS population and 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 *HIF1 $\alpha$*  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.<sup>57</sup> Therefore, rs142179458 is likely to be a potential loss-of-function variant, and mutant *HIF1 $\alpha$*  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.<sup>44</sup> With increasing dosages of *M. leprae* antigens (0, 5, and 20  $\mu\text{g}/\text{mL}$ ), 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;<sup>45</sup> 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;<sup>46</sup> 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.<sup>46</sup>

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.<sup>44</sup> 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.<sup>46</sup> 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<sup>14,40–42</sup> 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.

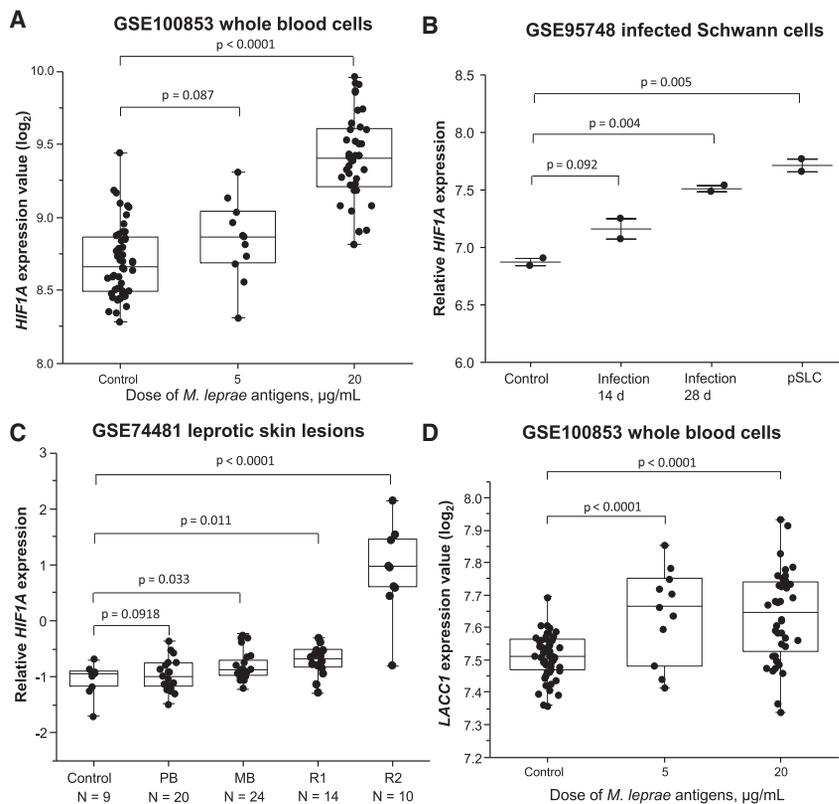
*CIITA* mRNA in whole blood cells after stimulation with a high concentration of *M. leprae* antigens (20  $\mu\text{g}/\text{mL}$ ;  $p = 0.004$ ; Figure S8B) and in mouse Schwann cells at the early stage of *M. leprae* infection (14 day;  $p = 0.043$ ) (Figure S8C). Together, the expression data indicate 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 suscepti-

bility genes and variants have been identified.<sup>9–11,14,16,58</sup> 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 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,<sup>44</sup> 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.<sup>45</sup> 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).<sup>45</sup>

(C) mRNA expression levels of *HIF1A* in leprotic skin lesions. The analysis was based on the microarray expression data GEO: GSE74481.<sup>46</sup> 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.<sup>44</sup> The p values were determined with Student's t test. Error bars represent the standard error of the mean.

(MIM: 604302).<sup>78</sup> A recent functional study showed that *LACC1* is a central regulator of metabolic function and bioenergetic state of macrophages<sup>79</sup> and could increase innate receptor-induced responses.<sup>80</sup> 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.<sup>81</sup> Disruption of *CIITA* might lead to dysregulation of *HLA* genes, which are main hits for genetic susceptibility to leprosy.<sup>14</sup> 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.<sup>58,82</sup> 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.<sup>58,82</sup> 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<sup>58</sup>). Evidently, large sample sizes are needed for further validation of our current results and the recently reported ones.<sup>58,82</sup>

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/>

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## Supplemental Data

### Missense Variants in *HIF1A* and *LACC1*

### Contribute to Leprosy Risk in Han Chinese

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## Supplemental Data

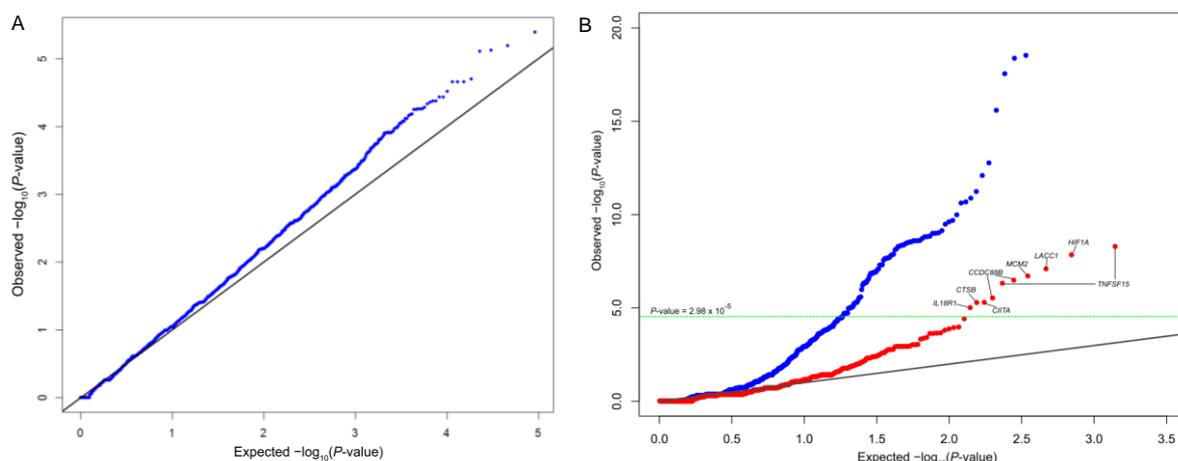


Figure S1. Quantile-quantile (Q-Q) plot showing the distribution of expected compared to observed  $-\log_{10}$  of  $P$ -values for the (A) exome sequencing results and (B) targeted next-generation sequencing results (blue dots) and the exclusion of the *HLA* genes (red dots). The plot was performed by using R (<https://www.r-project.org/>) with custom code. The x axis represents  $-\log_{10}$  expected  $P$  values, and the y axis represents  $-\log_{10}$  observed  $P$  values. The grey line represents  $y=x$ . The dashed green line ( $2.89 \times 10^{-5}$ ) indicates significant  $P$  value after multiple correction ( $0.05/1676$ ). The inflation factors ( $\lambda$ ) for the distribution of  $P$ -values were estimated to be 1.364 in the exome sequencing results (A) and 2.812 (including the *HLA* genes) and 1.277 (excluding the *HLA* genes) in targeted next-generation sequencing results (B), respectively, by using the GenABEL-package (estlambda, <http://www.genabel.org/manuals/GenABEL>). The decrease of  $\lambda$  indicates that it is reasonable to exclude the *HLA* genes in subsequent analyses. Note that the inflation factors were relatively high in this study, which could be explained by the following reasons: 1) the overall sample sizes for the exome sequencing and targeted sequencing were relatively small; 2) we selected these GWAS hit genes for the targeted sequencing, which were supposed to be more significant than the randomly selected genes; 3) the number of total SNPs used for making the Q-Q plot was limited, making the distribution to be potentially biased by several significant SNPs.

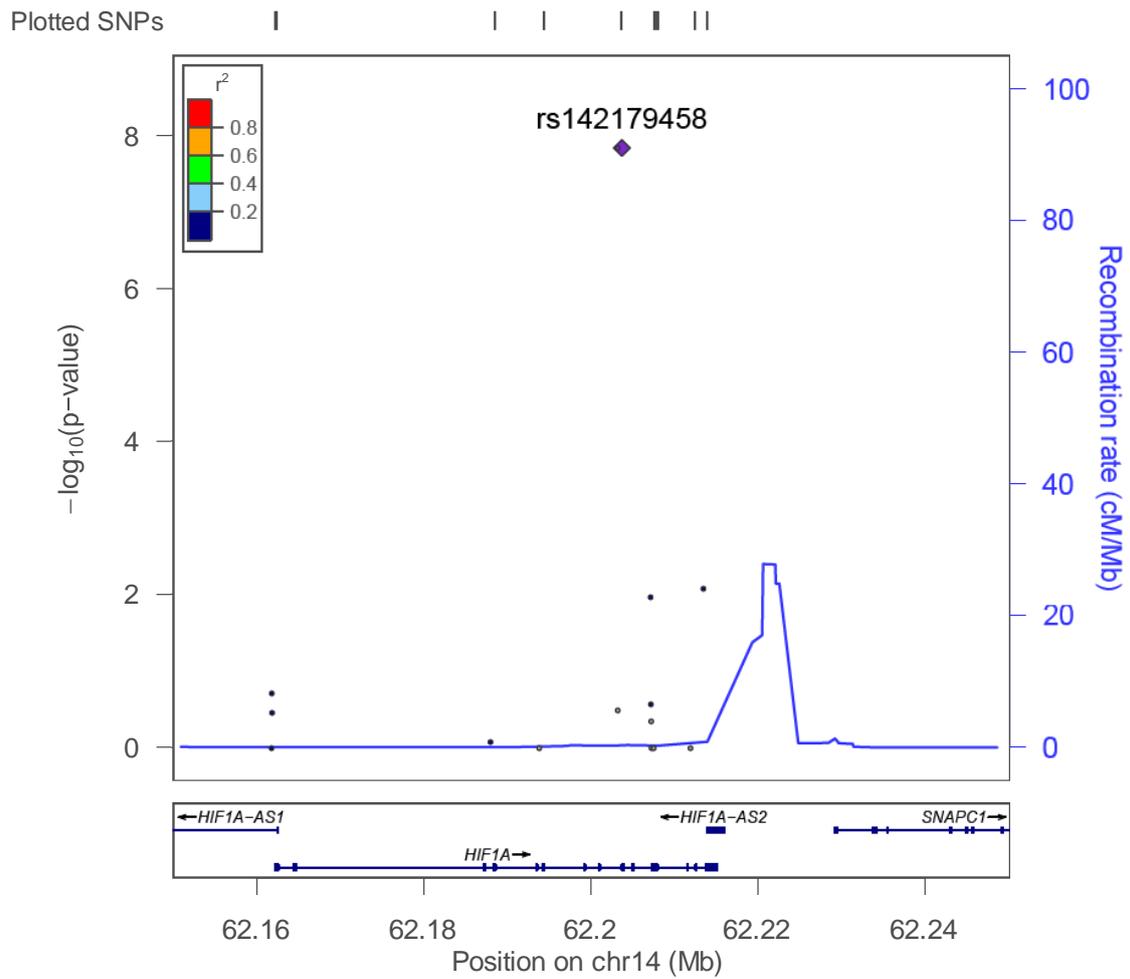


Figure S2. Regional association plot of *HIF1A* and surrounding region based on the targeted sequencing data in this study. SNP rs142179458 was significantly associated with leprosy, but this SNP was in low linkage disequilibrium (LD) with the surrounding SNPs. The plot was generated using the online tool LocusZoom (<http://locuszoom.sph.umich.edu/>). Genome Build /LD population was set as “hg19/1000 Genomes Nov2014 ASN”. Colors were determined by the LD between surrounding SNP and the most significant SNP rs142179458.

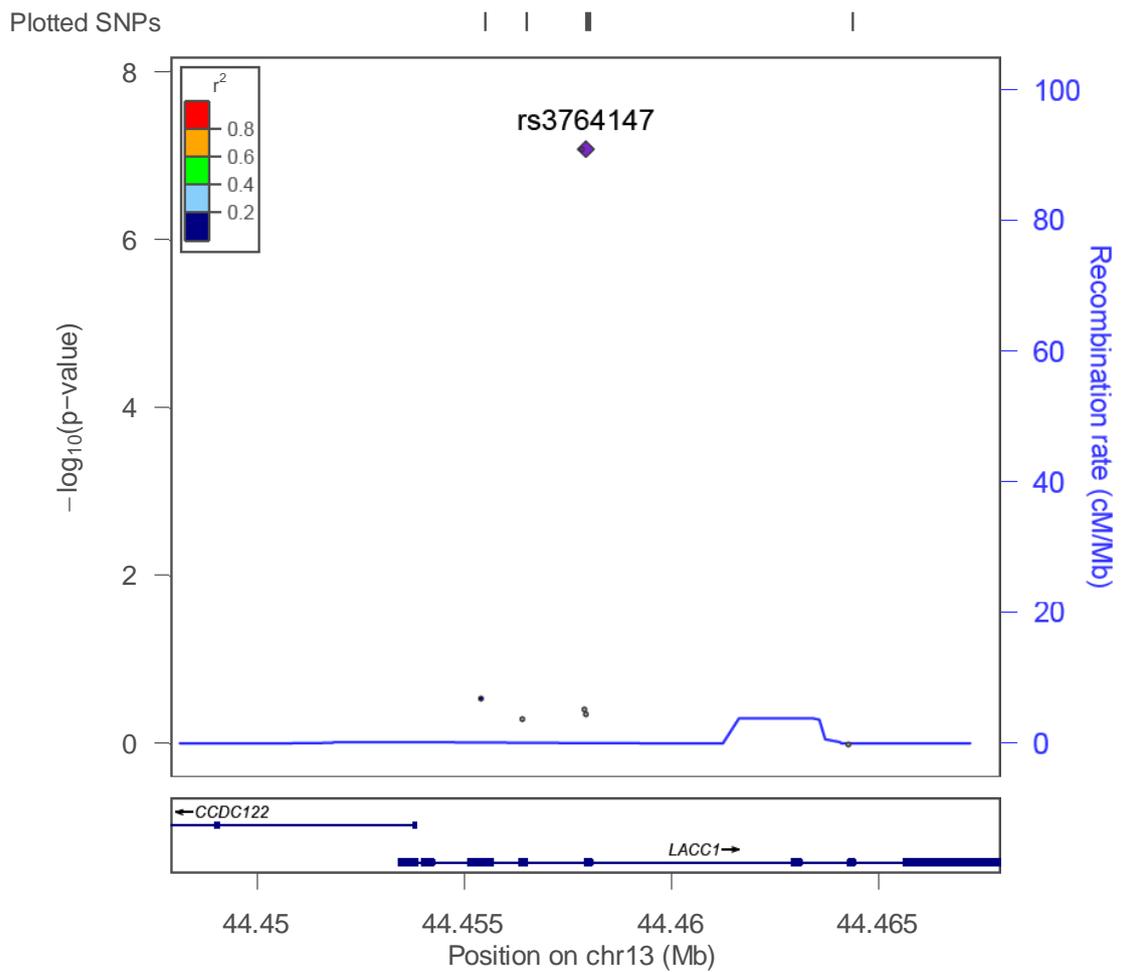


Figure S3. Regional association plot of *LACCI* and surrounding region based on the targeted sequencing data in this study. SNP rs3764147 was significantly associated with leprosy, but this SNP was in low linkage disequilibrium (LD) with the surrounding SNPs. The plot was generated using the online tool LocusZoom (<http://locuszoom.sph.umich.edu/>). Genome Build /LD population was set as “hg19/1000 Genomes Nov2014 ASN”. Colors were determined by the LD between surrounding SNP and the most significant SNP rs3764147.

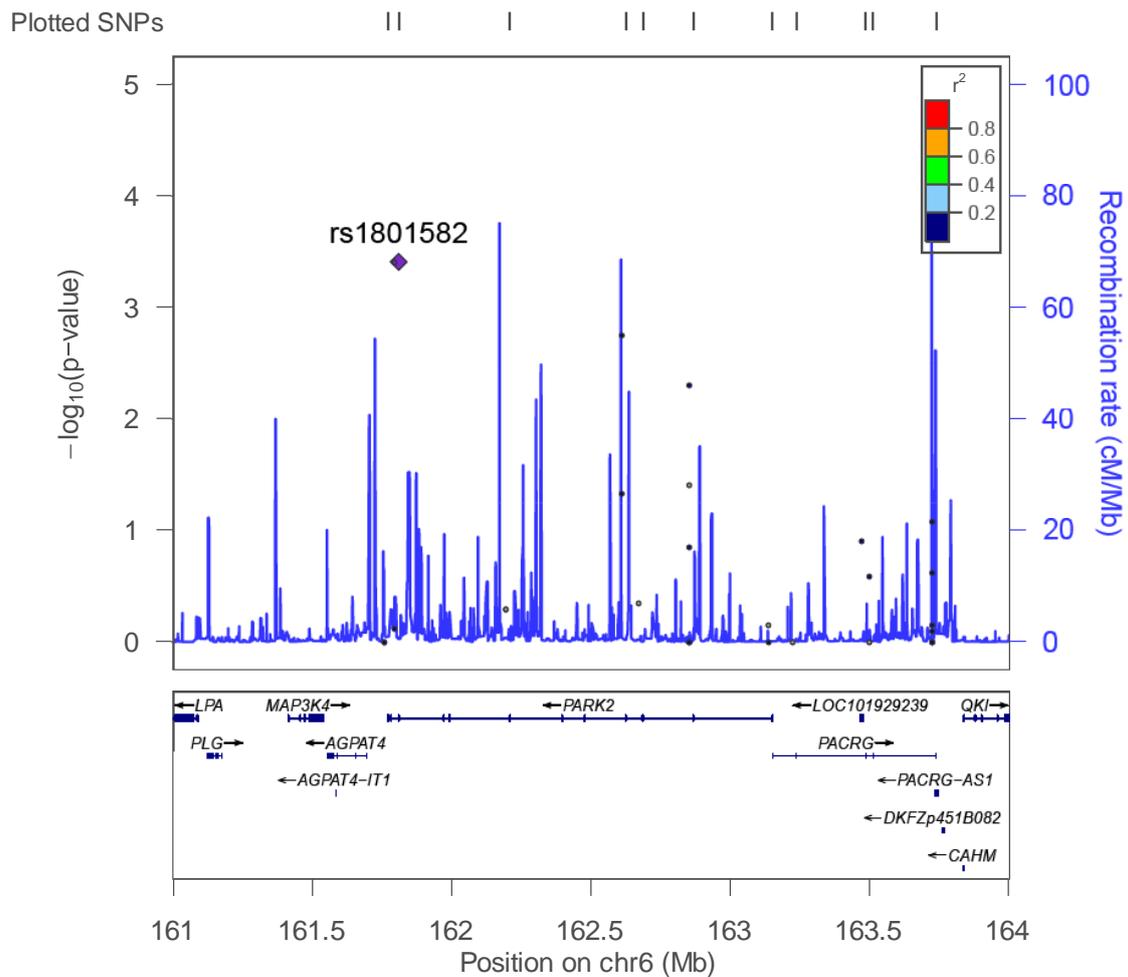


Figure S4. Regional association plot of the *PARK2/PRKN-PACRG* region and surrounding region based on targeted sequencing data in this study. The *PARK2/PRKN-PACRG* region was the first genome-wide significant region associated with leprosy identified by genome-wide linkage study (GWLS).<sup>1</sup> It is unknown which gene and which SNP might be the functional hit under the GWLS signal. In our deep sequencing of this region, we observed no functional SNPs significantly associated with leprosy. The most significant SNP in this region, a missense variant rs1801582 in *PARK2/PRKN*, showed a suggestive significance and was marked in the plot. The plot was generated using the online tool LocusZoom (<http://locuszoom.sph.umich.edu/>). Genome Build /LD population was set as “hg19/1000 Genomes Nov2014 ASN”. Colors were determined by the LD between surrounding SNP and the most significant SNP rs1801582.

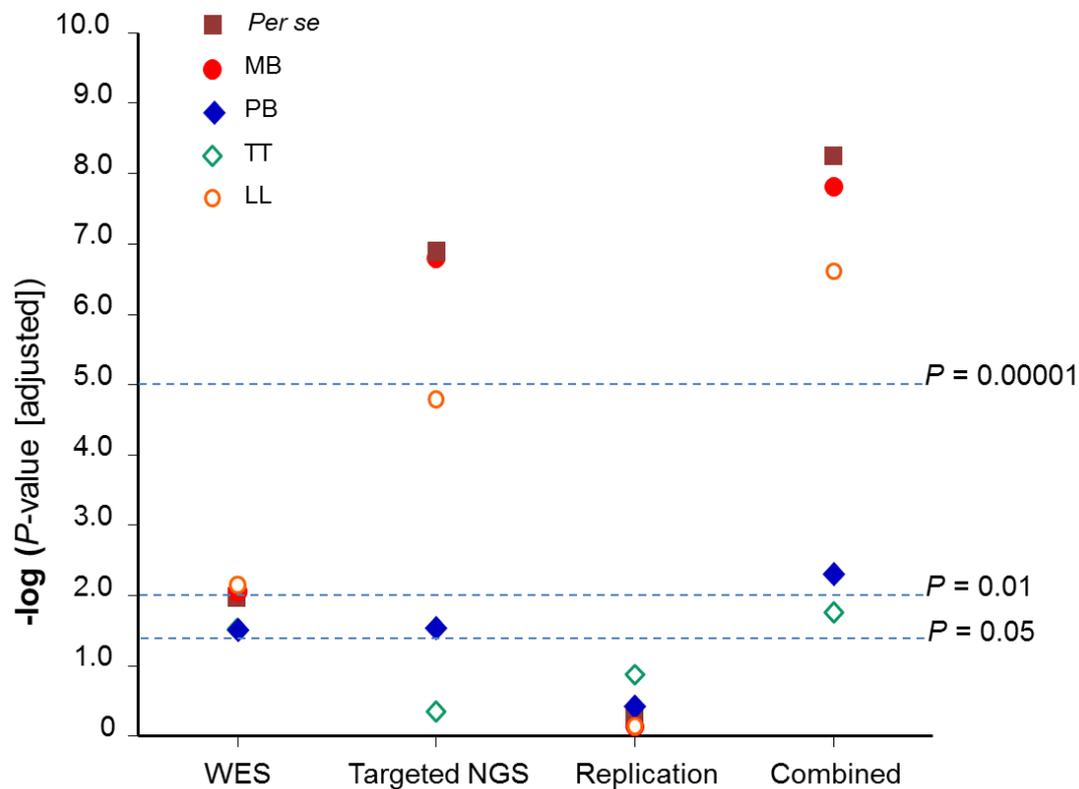


Figure S5. Statistical analyses of *HIF1A* rs142179458 between controls and leprosy *per se* and its clinical subtypes. The  $-\log(P\text{-values})$  were showed in our three-stage analysis. WES, samples (cases,  $n=108$ ; controls,  $n=52$ ) were analyzed by whole exome sequencing; Targeted NGS, samples (cases,  $n=798$ ; controls,  $n=990$ ) were analyzed by using targeted sequencing; Replication, the Yuxi sample (cases,  $n=527$ ; controls,  $n=583$ ) was used for replication; Combined, combined analysis for all samples of the three stages (cases,  $n=1,433$ ; controls,  $n=1,625$ ). *Per se* – Leprosy *per se*; MB – multibacillary leprosy; PB – paucibacillary leprosy; TT – tuberculoid; LL – lepromatous.

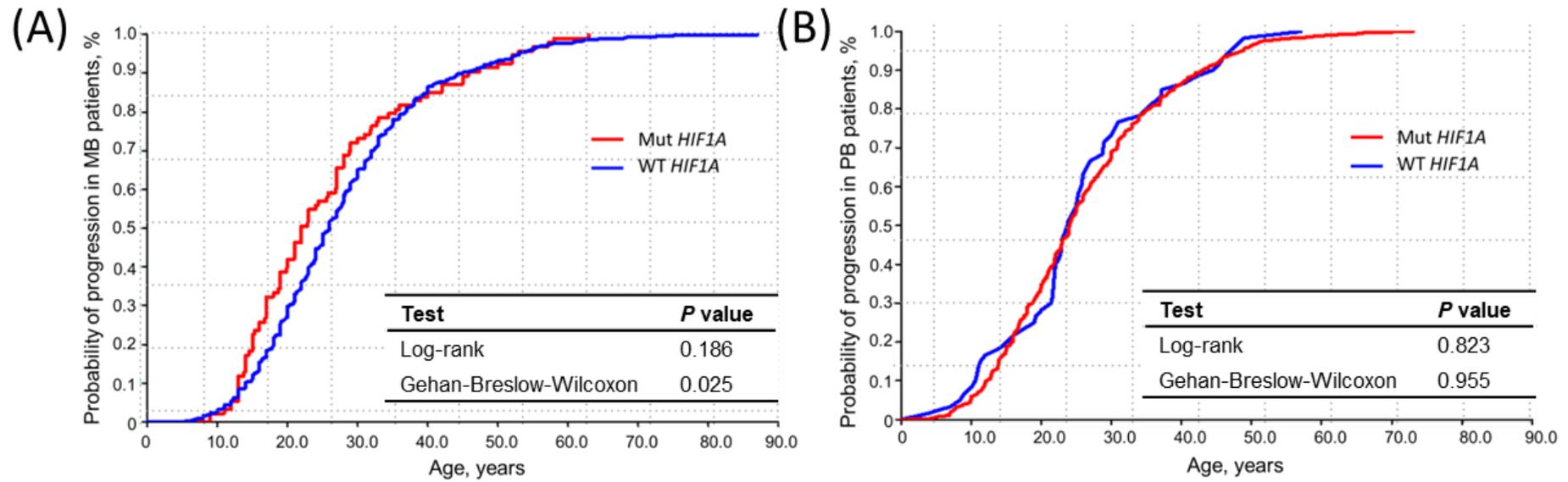
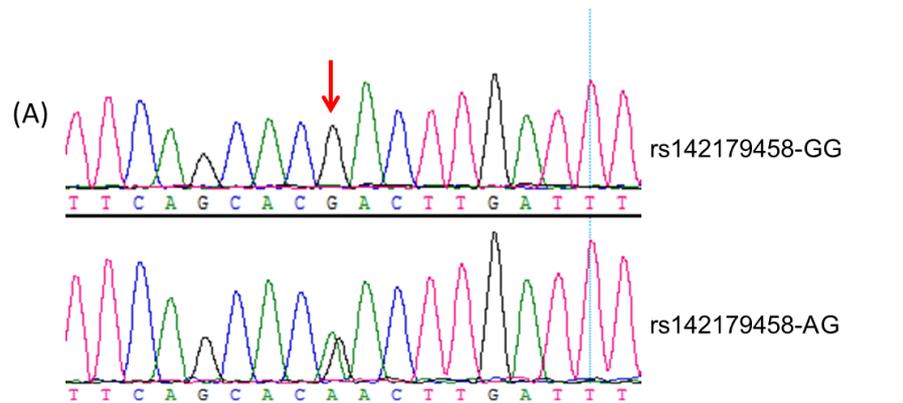


Figure S6. Carriers of *HIF1A* risk variant A of rs142179458 had an advanced leprosy onset age and this effect was restricted to individuals with multibacillary (MB), but not paucibacillary (PB) leprosy.



(B)

	p.D349
Human_ENST00000394997	SQPQCIVCVNYVSGIIQHDLIFSLQQTE
Gorilla_ENSGGOT0000028181	SQPQCIVCVNYVSGIIQHDLIFSLQQTE
Rhesus_ENSMUT0000029538	SQPQCIVCVNYVSGIIQHDLIFSLQQTE
Chimpanzee_ENSPTRT0000011753	SQPQCIVCVNYVSGIIQHDLIFSLQQTE
Treeshrew_TSDBT00022125	SQPQCIVCVNYVSGIIQHDLIFSLQQTE
Dog_ENSCAFT0000024897	SQPQCIVCVNYVSGIIQHDLIFSLQQTE
Cow_ENSBTAT0000027885	SQPQCIVCVNYVSGIIQHDLIFSLQQTE
Rat_ENSRNOT0000049725	SQPQCIVCVNYVSGIIQHDLIFSLQQTE
Rabbit_ENSOCUT0000017744	SQPQCIVCVNYVSGIIQHDLIFSLQQTE

Figure S7. Sanger sequencing of individuals harboring genotypes AG and GG of rs142179458 (A). Protein sequence alignments showing the conservation of Asp349 in 9 vertebrate species (B). The protein sequences were retrieved from ensembl (<http://asia.ensembl.org/index.html>)<sup>2</sup> and treeshewdb (<http://www.treeshewdb.org/>).<sup>3</sup> The sequence accession number is given after the species name.

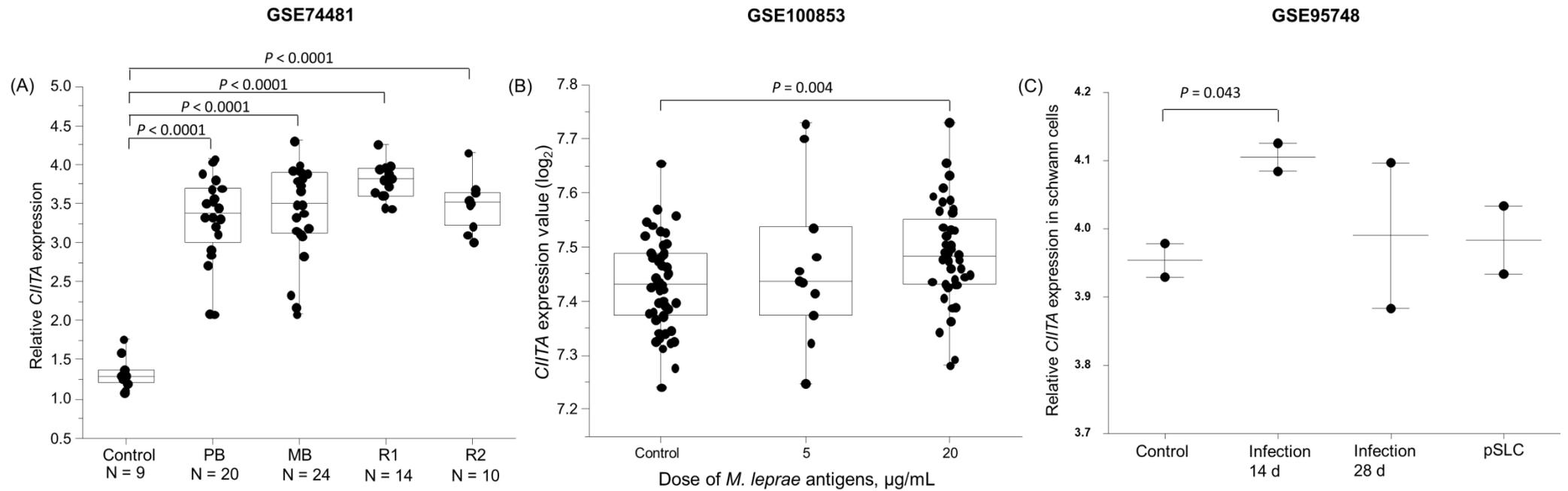


Figure S8. *CIITA* mRNA expression patterns in leprotic skin lesions and cells from individuals with leprosy stimulated by *M. leprae* sonicate. (A) mRNA expression level of *CIITA* in leprotic skin lesions was based on the microarray expression data GSE74481.<sup>4</sup> This dataset contains skin biopsies of 24 multibacillary (MB), 20 paucibacillary (PB), 14 type I reaction (R1) and 10 type II reaction (R2) individuals, as well as normal skin biopsies from 9 healthy individuals. (B) Expression analysis in whole blood cells of individuals with borderline leprosy stimulated with *M. leprae* sonicate. The expression dataset GSE100853,<sup>5</sup> which contains 51 unrelated individuals from Vietnam diagnosed with borderline leprosy, were used to determine the gene expression. Whole blood cells of each subject were stimulated with *M. leprae* sonicate (5  $\mu\text{g/mL}$  for 11 individuals and 20  $\mu\text{g/mL}$  for 40 individuals) or untreated (control). (C) *CIITA* mRNA expression in mouse Schwann cells infected with or

without *M. leprae*. The analysis was based on expression dataset GSE95748.<sup>6</sup> The Schwann cells were harvested at successive stages of the *M. leprae* infection for RNA extraction and hybridization using the Affymetrix microarrays. Three time points of infection were used in this study, including day 14 and day 28 after infection, and pSLC (progenitor/stem-like cells, leprosy bacteria trigger reprogramming of adult Schwann cells to a stage of pSLC<sup>6</sup>). The *P* values were determined using the Student's *t* test.

Table S1. Primers for genotyping

Gene	SNP ID	Primers (5'-3')	T <sub>m</sub> (°C)
<i>HIF1A</i>	rs142179458	F: TGTGACACAGTACGCATGA R: GGCTTG TAGCAACAGACA S: TCTTGAAATGTTCCCTGTCC	56
<i>LACC1</i>	rs3764147	F: TTTTGTGTTTTCCCATATATAA R: GGGCTAAAGACACTTAATCTG S: using forward primer	55
<i>CIITA</i>	rs199476072	F: GCTCACGGGACTCTATGTCGG R: ATGCCAGTGCTGCGGAGGT S: using reverse primer	60

F, forward primer; R, reverse primer; S, sequencing primer

Table S2. List of genes located in the published genome-wide significant loci that were analyzed by the targeted sequencing in this study

<b>Genes</b>	<b>Proxy SNP</b>	<b>Reference</b>
<i>PARK2-PACRG</i>	-	Mira et al. <sup>1</sup>
<i>HLA-DRB5, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DQB2, HLA-B</i>	rs602875	Zhang et al. <sup>7</sup>
<i>RIPK2</i>	rs42490	Zhang et al. <sup>7</sup>
<i>TNFSF15, TNFSF8</i>	rs6478108	Zhang et al. <sup>7</sup>
<i>LRRK2</i>	rs1873613	Zhang et al. <sup>7</sup>
<i>CCDC122</i>	rs3088362	Zhang et al. <sup>7</sup>
<i>LACCI/C13orf31</i>	rs3764147	Zhang et al. <sup>7</sup>
<i>NOD2</i>	rs9302752	Zhang et al. <sup>7</sup>
<i>ALS2CL</i>	rs4076927	Zhang et al. <sup>7</sup>
<i>GNG2</i>	rs10133203	Zhang et al. <sup>7</sup>
<i>IL23R</i>	rs3762318	Zhang et al. <sup>8</sup>
<i>RAB32</i>	rs2275606	Zhang et al. <sup>8</sup>
<i>BATF3</i>	rs2221593	Liu et al. <sup>9</sup>
<i>CDH18</i>	rs73058713	Liu et al. <sup>9</sup>
<i>DECI (failed)</i>	rs10817758	Liu et al. <sup>9</sup>
<i>EGR2</i>	rs58600253	Liu et al. <sup>9</sup>
<i>CCDC88B</i>	rs663743	Liu et al. <sup>9</sup>
<i>CIITA, SOCS1</i>	rs77061563	Liu et al. <sup>9</sup>
<i>COX4I1</i>	rs2733954	Liu et al. <sup>9</sup>
<i>IL1RL1, IL18RAP, IL18R1</i>	rs76886731	Liu et al. <sup>9</sup>
<i>BCL10</i>	rs817462	Liu et al. <sup>9</sup>
<i>IL12B</i>	rs6863015	Liu et al. <sup>9</sup>
<i>SYN2, PPARG</i>	rs6807915	Wang et al. <sup>10</sup>
<i>BBS9 (failed)</i>	rs4720118	Wang et al. <sup>10</sup>
<i>CTSB</i>	rs55894533	Wang et al. <sup>10</sup>
<i>MED30 (failed)</i>	rs10100465	Wang et al. <sup>10</sup>
<i>IL4R</i>	rs34411505	Wang et al. <sup>10</sup>

Table S3. Population attributable fraction (PAF) estimation for *HIF1A* rs142179458, *LACC1* rs3764147, and reported genome-wide significant loci

Gene	SNP	Function	Reference	Frequency	PAF
<i>HLA-DR-DQ</i>	rs602875	intron	Zhang et al. <sup>7</sup>	0.680	25.089
<i>RIPK2</i>	rs42490	intron	Zhang et al. <sup>7</sup>	0.580	15.480
<i>TNFSF15</i>	rs6478108	intron	Zhang et al. <sup>7</sup>	0.460	14.545
<i>LRRK2</i>	rs1873613	intron	Zhang et al. <sup>7</sup>	0.750	10.881
<i>CCDC122</i>	rs3088362	intron	Zhang et al. <sup>7</sup>	0.260	11.910
<i>LACC1</i>	rs3764147	<b>missense</b>	Zhang et al. <sup>7</sup>	0.310	17.410
<i>NOD2</i>	rs9302752	intron	Zhang et al. <sup>7</sup>	0.290	14.610
<i>IL23R</i>	rs3762318	intron	Zhang et al. <sup>8</sup>	0.900	28.793
<i>RAB32</i>	rs2275606	5upstream	Zhang et al. <sup>8</sup>	0.210	5.927
<i>BATF3</i>	rs2221593	5upstream	Liu et al. <sup>9</sup>	0.199	2.977
<i>CDH18</i>	rs73058713	intron	Liu et al. <sup>9</sup>	0.145	2.698
<i>DEC1</i>	rs10817758	intron	Liu et al. <sup>9</sup>	0.381	4.829
<i>EGR2</i>	rs58600253	intron	Liu et al. <sup>9</sup>	0.145	3.090
<i>CCDC88B</i>	rs663743	utr-5	Liu et al. <sup>9</sup>	0.146	3.339
<i>CIITA</i>	rs77061563	intron	Liu et al. <sup>9</sup>	0.623	10.822
<i>COX4I1</i>	rs2733954	intron	Liu et al. <sup>9</sup>	0.736	8.836
<i>BCL10</i>	rs817462	intron	Liu et al. <sup>9</sup>	0.294	1.945
<i>IL18R1</i>	rs76886731	intron	Liu et al. <sup>9</sup>	0.483	10.420
<i>IL12B</i>	rs6863015	intron	Liu et al. <sup>9</sup>	0.697	14.183
<i>SYN2</i>	rs6807915	intron	Wang et al. <sup>10</sup>	0.500	5.820
<i>BBS9</i>	rs4720118	intron	Wang et al. <sup>10</sup>	0.310	4.726
<i>CTSB</i>	rs55894533	intron	Wang et al. <sup>10</sup>	0.430	6.059
<i>MED30</i>	rs10100465	intron	Wang et al. <sup>10</sup>	0.710	11.134
<i>IL4R</i>	rs34411505	intron	Wang et al. <sup>10</sup>	0.840	12.029
<i>NCKIPSD</i>	rs145562243	<b>missense</b>	Liu et al. <sup>11</sup>	0.002	0.500
<i>CARD9</i>	rs149308743	<b>missense</b>	Liu et al. <sup>11</sup>	0.001	0.448
<i>IL23R</i>	rs76418789	<b>missense</b>	Liu et al. <sup>11</sup>	0.048	1.741
<i>FLG</i>	rs146466242	<b>missense</b>	Liu et al. <sup>11</sup>	0.040	1.768
<i>USP49</i>	rs75746803	<b>missense</b>	Liu et al. <sup>11</sup>	0.050	1.381
<i>TYK2</i>	rs55882956	<b>missense</b>	Liu et al. <sup>11</sup>	0.038	1.098
<i>LACC1</i>	rs3764147	<b>missense</b>	This study	0.299	15.318
<i>HIF1A</i>	rs142179458	<b>missense</b>	This study	0.027	3.439

PAF was calculated to determine the effect of a risk factor on leprosy incidence in a population based on odds ratio and allele frequency using the equation

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

in which  $P$  is the frequency of the risk allele in the general population, and OR is the odds ratios of the risk allele in previous reported GWAS and/or current study.

## Supplemental References

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