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A pleiotropic effect of the *APOE* gene: association of *APOE* polymorphisms with multibacillary leprosy in Han Chinese from Southwest China*

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Summary

Background Patients with leprosy have a very low risk of Alzheimer disease (AD) and β-amyloid (Aβ) deposition is significantly lower in the brain tissue of elderly patients with leprosy compared with age-matched controls. Apolipoprotein E (ApoE) plays a critical role in lipid metabolic pathways and in the brain, facilitating the proteolytic clearance of Aβ. We hypothesized that *APOE* confers risk of leprosy as lipid metabolism is involved in *Mycobacterium leprae* infection.

Objectives To investigate the potential genetic associations between *APOE* and leprosy in two independent Chinese case–control cohorts from the Yuxi and Wenshan prefectures, Yunnan Province of Southwest China.

Methods Five *APOE* single-nucleotide polymorphisms (SNPs) were analysed in 1110 individuals (527 patients and 583 controls) from the Yuxi prefecture using a SNaPshot assay. Genetic variations in the entire *APOE* exons were screened in 1788 individuals (798 patients and 990 controls) from the Wenshan prefecture using next-generation sequencing technology.

Results The AD-associated SNPs rs405509 and rs439401 increased the risk of leprosy per se and multibacillary leprosy ($P < 0{\cdot}005$), but the *APOE*-ε4 allele did not. The SNPs rs405509 and rs439401 were *cis* expression quantitative trait loci (eQTL) for *APOE* expression in human skin. Differential *APOE* mRNA expression was observed in skin lesions of patients with type I reaction leprosy and those with multibacillary leprosy. *APOE* and related lipid genes are involved in an interaction network with leprosy susceptibility genes.

Conclusions The *APOE* gene is associated with leprosy, most likely by regulating lipid-metabolism-related genes.

What's already known about this topic?

- Host genetic factors could influence susceptibility to leprosy, which is a chronic infectious and neurological disease.
- Previous studies have reported a significantly lower level of β-amyloid (Aβ) deposition in the brain tissues of elderly people with leprosy compared with age-matched controls.
- Apolipoprotein E (ApoE) facilitates proteolytic clearance and homeostasis of Aβ peptides in the brain.

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Conflicts of interest

None declared.

**Plain language summary available online*

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What does this study add?

- Common variants of *APOE* were associated with multibacillary leprosy in Han Chinese.
- The risk single-nucleotide polymorphisms rs405509 and rs439401 were *cis* expression quantitative trait loci for *APOE* expression in human skin.
- ApoE may interact with proteins that are coded as leprosy-risk genes.

What is the translational message?

- Our findings suggested that *APOE* is associated with leprosy, and this might be mediated by the altered expression of *APOE* and its potential role in lipid metabolic pathways during *Mycobacterium leprae* infection.
- Future studies that explore the potential function of *APOE* during leprosy onset may offer a novel therapeutic target in leprosy.

Leprosy is a chronic infectious and neurological disease caused by the obligate intracellular bacillus *Mycobacterium leprae*.[1] *M. leprae* has a tropism for macrophages and Schwann cells, and results in inflammatory responses and nerve damage.[2] Patients with leprosy show a wide spectrum of clinical types based on host immune status and response, ranging from lepromatous (LL) to tuberculoid (TT) leprosy.[3] The LL type is characterized by widespread skin lesions, where numerous bacilli live in foamy histiocytes that are filled with lipids.[4,5] For treatment purposes, leprosy has been categorized as paucibacillary (PB) and multibacillary (MB) leprosy by the World Health Organization.[6] Leprosy affects more than 200 000 people (with 210 758 new cases in 2015), mainly in India, Brazil and Indonesia[7] and is still considered as a public health problem.

The pathogenic mechanisms involved in leprosy are still unknown. Currently, increasing evidence suggest that host genetic factors could influence susceptibility to leprosy.[8,9] Genome-wide linkage studies have identified many chromosome regions (e.g. 10p13,[10] 6q25[11] and 20p12[12]) that are associated with leprosy. A previously published genome-wide association study (GWAS) in Han Chinese populations has reported many leprosy susceptibility genes *CCDC122*, *LACC1*, *NOD2*, *TNFSF15*, *HLA-DR*, *RIPK2* and *LRRK2*.[13] In addition, more than 60 susceptibility genes were identified using a candidate gene approach, such as *NOD2*,[14] *VDR*, *MBL*, *TNF*,[15] *MRC1*,[16] *IFNG*,[17] *MBL2*,[18,19] *TLR1*,[20] *TOLLIP*,[21] *PINK1* and *PARL*.[22]

The *APOE* gene is located in 19q13 and encodes a member of the family of soluble apolipoproteins. It plays an important role in lipid (triglycerides and cholesterol) transport and delivery, as well as in lipoprotein metabolism.[23] *APOE* has three common isoforms: *APOE*-ε2 [Cys-112, Cys-158 (also named Cys-130, Cys-176 when the 18-aa signalling peptide is included)], *APOE*-ε3 (Cys-112, Arg-158) and *APOE*-ε4 (Arg-112, Arg-158), which affects brain function.[24,25] Previous studies had shown that *APOE*-ε4 confers the greatest risk for Alzheimer disease (AD),[26] stroke[27] and coronary heart disease.[28] Given that patients with leprosy have a very low risk of AD[29] and that infection with *M. leprae* affects lipid metabolism,[30,31] we hypothesized that the AD-risk *APOE* allele might have a low frequency in people with leprosy. In this study, we investigated whether *APOE* genetic variants are associated with leprosy in Han Chinese from Southwest China. We integrated expression quantitative trait loci (eQTL), mRNA expression and protein interaction analysis to help establish the potential role of *APOE* in leprosy.

Materials and methods

Participants

The data of 2898 individuals from the Yuxi and Wenshan prefectures, Yunnan Province of Southwest China were analysed in this study. The Yuxi samples have been described previously.[17] In brief, samples from 527 patients with leprosy and 583 healthy controls were collected from the same geographic region. The Wenshan data contained 798 patients with leprosy and 990 healthy controls. All patients were diagnosed based on clinical and histopathological features and/or bacteriological index (if available), as described in our previous study.[32] We determined the clinical subtype of leprosy using Chen *et al.*'s[33] strategy and grouped the cases into MB and PB leprosy.

Information about each patient included family history, age at onset, bacteriological and histopathological profile (if available), disability level and cure or relapse condition after treatment. The regionally matched healthy individuals had no history of leprosy, HIV infection and tuberculosis. All leprosy patients had received antileprosy drug treatment at the time of sample collection (Table 1). Written informed consent conforming to the tenets of the Declaration of Helsinki were obtained from each participant prior to the study. The institutional review board of the Kunming Institute of Zoology approved all the experimental protocols of this study.

Single-nucleotide polymorphism selection and genotyping

Genomic DNA was extracted from whole blood using the AxyPrep™ Blood Genomic DNA Miniprep Kit (Corning Inc., Corning, NY, U.S.A.). Three tag single-nucleotide

Table 1 Clinical and demographic information for the patients with leprosy and healthy controls from Yunnan Province of Southwest China

Item	Yuxi sample	Wenshan sample[a]
Patients with leprosy		
n	527	798
Age at onset, years: mean ± SD	24·8 ± 12·4	26·5 ± 12·5
Female	140 (26·6)	251 (31·4)
Multibacillary leprosy	279 (52·9)	452 (59·2)
Multidrug therapy	165 (31·3)	441 (61·2)
Controls		
n	583	990
Age, years: mean ± SD	36·0 ± 15·5	38·1 ± 14·0
Female	219 (37·6)	439 (44·3)

Data are n (%) unless otherwise specified. [a]In the Wensan sample, 35 patients with leprosy had missing information regarding multibacillary and paucibacillary leprosy classification and 77 patients had missing information for antileprosy treatment. These patients were excluded for computing the relevant percentages.

polymorphisms (SNPs) of *APOE* (rs405509, rs769450 and rs439401) were selected according to the linkage disequilibrium (LD) pattern of the HapMap CHB (Han Chinese in Beijing) dataset (Phase 3; International HapMap Consortium).[34] Two nonsynonymous SNPs [rs429358 (p.C130R) and rs7412 (p.R176C)] that were associated with AD were also included in the analysis. SNPs rs405509, rs769450 and rs439401 were genotyped using the SNaPshot assay (Thermo Fisher Scientific, Waltham, MA, U.S.A.) following the procedure described in our previous studies,[17,22] whereas rs429358 and rs7412 were genotyped by direct sequencing using the ABI 3730xl DNA Analyzer (Thermo Fisher Scientific). The primers used for genotyping are shown in Table S1 (see Supporting Information). The exons and flanking regions of *APOE* were captured using the NimbleGene SeqCap EZ Human Exome v3.0 (Roche, Pleasanton, CA, U.S.A.) and sequenced by the Illumina HiSeq 4000 Genome Analyzer (Illumina, San Diego, CA, U.S.A.). The alignment and variant calling for the *APOE* exons was performed using the same procedure described in our previous study.[22]

Expression quantitative trait loci and differential mRNA expression analysis

We performed eQTL analysis to investigate whether the *APOE* variants affect gene expression in human whole blood and skin tissue using the publicly available Genotype-Tissue Expression dataset (GTEx, http://www.gtexportal.org/home/).[35] We reanalysed microarray data (Gene Expression Omnibus accession number GSE74481)[36] regarding leprosy skin lesions, including 24 patients with MB [10 mid-borderline leprosy (BB), 10 borderline lepromatous (BL) and 4 LL], 20 with PB [10 TT and 10 borderline-tuberculoid (BT)], 14 with a type I reaction (R1) and 10 with a type II reaction (R2) leprosy, and normal skin biopsies from nine healthy individuals. The patients with nonreactional leprosy had received no treatment at the time of diagnosis, whereas the patients with R1 and R2 had received leprosy treatment.[36]

Protein interaction network analysis

To establish the potential involvement of apolipoprotein E (ApoE) in leprosy, we first constructed an interaction network for *APOE* that included 19 *APOE*-interactive lipid genes (encoding proteins in lipid metabolic processes, lipoprotein transportation and lipid metabolism) from the STRING database (http://string-db.org)[37] and 227 published leprosy-risk genes (Zhang *et al.*[9] and references therein). We used the high-confidence protein interaction webserver GeneMANIA (http://www.genemania.org/)[38] to construct potential interactions among these genes. As the resulting network was too complex (data not shown), we retrieved hub genes (each gene occupies a hub node in the network and has 40 connections or more) using an in-house Perl script. We then reconstructed the interaction network for these hub genes using GeneMANIA.[38] This simplified network of hub genes keeps the conserved signal from the original interaction network and offers a clearer view of the interactions of *APOE* and related key genes.

Statistical analysis

Cases and controls were compared on the basis of the frequencies of genotypes and alleles. LD structure was determined using Haploview 4.2 (Broad Institute, Cambridge, MA, U.S.A.).[39] For deviation from the Hardy–Weinberg equilibrium (HWE), haplotype comparisons were performed using PLINK v1.07 (www.cog-genomics.org/plink/1.9/).[40] Power calculations were performed using Quanto software (USC Biostats, Los Angeles, CA, U.S.A.).[41] A P-value less than 0·05 was considered as statistically significant. Bonferroni corrected P-values were used for multiple comparisons.

Results

Association of *APOE* single-nucleotide polymorphisms with leprosy per se and multibacillary subtype

All the SNPs were in Hardy–Weinberg equilibrium in controls ($P > 0·05$). We constructed the LD map of these SNPs in the Yuxi leprosy cases and Yuxi controls, and observed similar LD structures for the two populations (Fig. S1; see Supporting Information). Based on the minor allele frequencies (MAF) of the five *APOE* SNPs [rs405509, rs769450, rs439401, rs429358 (p.C130R) and rs7412 (p.R176C)] in the studied populations (Table 2), our study had sufficient power for these SNPs (MAF > 0·27) of modest effect [odds ratio (OR) > 1·4] (Fig. S2; see Supporting Information).

Two SNPs showed an association with leprosy per se (rs405509, $P_{genotype} = 0·005$, $P_{dominant} = 0·002$; and

Table 2 Comparison of the allele and genotype frequencies of five *APOE* single-nucleotide polymorphisms (SNPs) in 527 patients with leprosy and 583 healthy controls from the Yuxi prefecture, Yunnan Province of Southwest China

			Leprosy per se vs. controls			MB vs. controls			PB vs. controls		
SNP/model	Allele/genotype	Controls, n[a]	n[a]	P[b]	OR (95% CI)	n[a]	P[b]	OR (95% CI)	n[a]	P[b]	OR (95% CI)
rs405509											
Genotypes	CC/CA/AA	47/222/314	42/249/234	**0·005**	–	23/134/121	0·013	–	19/115/113	0·070	–
Allele	C/A	0·271	0·317	0·017	NS	0·324	0·024	NS	0·310	0·109	NS
Dominant	CC+CA/AA	269/314	291/234	**0·002**	1·452 (1·145–1·840)	157/121	**0·005**	1·515 (1·136–2·019)	134/113	0·033	NS
Recessive	CC/CA+AA	47/536	42/483	0·970	NS	23/255	0·915	NS	19/228	0·857	NS
rs769450											
Genotypes	AA/AG/GG	26/157/400	21/179/325	0·035	–	11/99/168	0·034	–	10/80/157	0·281	–
Allele	A/G	0·179	0·211	0·063	NS	0·218	0·058	NS	0·202	0·267	NS
Dominant	AA+AG/GG	183/400	200/325	0·019	NS	110/168	0·018	NS	90/157	0·157	NS
Recessive	AA/AG+GG	26/557	21/504	0·705	NS	11/267	0·734	NS	10/237	0·790	NS
rs429358											
Genotypes	CC/CT/TT	4/101/478	4/104/418	0·567	–	2/52/224	0·882	–	2/52/194	0·451	–
Allele	C/T	0·090	0·106	0·308	NS	0·101	0·633	NS	0·113	0·226	NS
Dominant	CC+CT/TT	105/478	108/418	0·287	NS	54/224	0·617	NS	54/194	0·207	NS
Recessive	CC/CT+TT	4/579	4/522	0·884	NS	2/276	0·956	NS	2/246	0·851	NS
rs7412											
Genotypes	TT/TC/CC	1/85/497	0/89/437	0·364	–	0/46/232	0·598	–	0/43/205	0·491	–
Allele	T/C	0·075	0·085	0·385	NS	0·083	0·555	NS	0·087	0·401	NS
Dominant	TT+TC/CC	86/497	89/437	0·323	NS	46/232	0·494	NS	43/205	0·346	NS
Recessive	TT/TC+CC	1/582	0/526	–	–	0/278	–	–	0/248	–	–
rs439401											
Genotypes	CC/CT/TT	98/255/230	89/284/154	**0·001**	–	43/157/79	**0·002**	–	46/127/75	0·040	–
Allele	C/T	0·387	0·438	0·014	NS	0·436	0·054	NS	0·442	0·037	NS
Dominant	CC+CT/TT	352/230	373/154	**$3{\cdot}200 \times 10^{-4}$**	1·583 (1·232–2·034)	200/79	**0·001**	1·654 (1·215–2·253)	173/75	0·012	NS
Recessive	CC/CT+TT	98/485	89/438	0·972	NS	43/236	0·604	NS	46/202	0·545	NS

MB, multibacillary leprosy; PB, paucibacillary leprosy; OR, odds ratio; CI, confidence interval; NS, not significant. [a]The minor allele frequency (MAF) is shown in the allele distribution; [b]P-value < 0·01 (0·05/5) was set as statistically significant after Bonferroni correction and P-values < 0·01 are in bold.

rs439401, $P_{genotype} = 0{\cdot}001$, $P_{dominant} = 3{\cdot}200 \times 10^{-4}$) and MB (rs405509, $P_{dominant} = 0{\cdot}005$; and rs439401, $P_{genotype} = 0{\cdot}002$, $P_{dominant} = 0{\cdot}001$) at the genotypic levels (Table 2). As the cases and controls were not matched for sex in our sample, we also performed association analyses using unconditional logistic regression with an adjustment for sex. The association of rs405509 and rs439401 with leprosy per se and MB ($P < 0{\cdot}01$) was replicated, both at the allelic and genotype level after Bonferroni correction (Table S2; see Supporting Information).

Haplotypes were reconstructed for the five SNPs (rs405509–rs769450–rs429358–rs7412–rs439401), two leprosy-associated SNPs (rs405509–rs439401) and two AD-associated risk SNPs (rs429358–rs7412). We found that only the haplotypes of the two leprosy-associated SNPs (rs405509–rs439401) showed a significantly different haplotype distribution pattern in the cases and controls (A-T, $P = 0{\cdot}012$; C-C, $P = 0{\cdot}015$; Table S3; see Supporting Information) and the difference survived Bonferroni correction for multiple comparisons.

Deep sequencing of the *APOE* exons to identify potential coding risk variants

To identify whether there are any other rare (allele frequency < 1%) or common variants (not captured by the tag SNPs) in the *APOE* gene that would confer risk of leprosy, we performed targeted gene sequencing for this gene (including the entire exons and the flanking regions) in 798 patients with leprosy patients and 990 healthy controls from the Wenshan prefecture, and compared this with East Asian (EAS) data from the Exome Aggregation Consortium (ExAC) dataset (http://exac.broadinstitute.org/).[42] In total, 59 rare variants (MAF < 1%) and three common variants (MAF > 1%: rs440446, rs429358 and rs7412) were identified (Table 3 and Table S4; see Supporting Information). The SNPs rs440446 and rs429358 were associated with leprosy when we compared the patients with leprosy with the healthy controls, but the significance did not survive Bonferroni correction (Table 3).

As the leprosy-associated SNPs (rs405509 and rs439401) identified in the Yuxi sample were not covered by target sequencing, we checked the LD pattern of the five SNPs (rs405509, rs439401, rs440446, rs429358 and rs7412) in the CHB population from the 1000 Genomes dataset.[43] We found that rs440446 was linked with rs439401 ($r^2 = 0{\cdot}86$). The SNP rs440446 in the Wenshan sample had an OR in the same direction (OR 1·193, 95% confidence interval 1·045–1·363, $P = 0{\cdot}010$) as that of rs439401 in the Yuxi sample, providing further evidence for the association of *APOE* SNPs with leprosy.

The leprosy-risk single-nucleotide polymorphisms affected *APOE* expression in human tissue

Next, we tested the eQTL effect of the five SNPs (rs405509, rs769450, rs429358, rs7412 and rs439401) genotyped in the

Table 3 Common single-nucleotide polymorphisms (SNPs) in the exon and flanking regions of the *APOE* gene in 798 patients with leprosy and 990 controls from the Wenshan prefecture, Yunnan Province of Southwest China[a]

Position	SNP[b]	Function	Ref	Alt	Residue change	Damaging prediction[c]	Allele counts in patients	Allele counts in controls	p[d]	OR (95% CI)	Allele counts in ExAC-EAS[e]	p[d]	OR (95% CI)
Chr19:45409167	rs440446	intron	C	G	–	Tolerated	745/1588	839/1972	0·010	1·193 (1·045–1·363)	206/474	0·190	1·150 (0·935–1·414)
Chr19:45411941	rs429358	missense	T	C	p.C130R	Tolerated	202/1596	194/1980	0·007	1·334 (1·082–1·644)	157/1044	0·082	0·819 (0·654–1·025)
Chr19:45412079	rs7412	missense	C	T	p.R176C	Damaging	120/1590	170/1962	0·242	0·861 (0·674–1·098)	34/418	0·680	0·922 (0·620–1·371)

Chr, chromosome; Ref, reference allele; Alt, alternative allele; OR, odds ratio; CI, confidence interval. [a]A variant with a frequency < 1% in either the patient or control group was regarded as a rare variant. [b]Among the five SNPs genotyped in this study, two missense variants were captured in the exome sequencing and are in italic. [c]Missense variants are rated as damaging when at least two of five prediction algorithms (SIFT,[62,63] PolyPhen2 HumDiv, PolyPhen2 HumVar,[64] LRT[65] and MutationTaster[66]) suggesting a potential deleterious effect; otherwise the variants are rated as tolerated; [d]P-values were calculated using Fisher's exact test. All P-values were not statistically significant after Bonferroni correction. [e]ExAC-EAS: 4327 East Asian (EAS) individuals in the ExAC database.[42]

Yuxi sample and the three common SNPs (rs440446, rs429358 and rs7412) identified in the Wenshan sample in human blood and skin tissue using the dataset from the GTEx project.[35] We found that the two leprosy-risk SNPs in the Yuxi sample were significant *cis* eQTL (rs405509, $P = 3{\cdot}80 \times 10^{-6}$, Fig. 1a; rs439401, $P = 3{\cdot}40 \times 10^{-12}$, Fig. 1b) in skin tissue. In addition, we found a significantly differential mRNA expression of *APOE* in skin lesion of R1 leprosy ($P_{adjusted} = 1{\cdot}17 \times 10^{-4}$) and a slightly changed expression in patients with MB leprosy ($P_{adjusted} = 0{\cdot}017$) compared with those of healthy controls from the dataset GSE74481 (Fig. 1c);[36] however, it should be noted that this observation was based on a limited number of samples and should be viewed with caution.

Apolipoprotein E could interact with leprosy-risk genes

To discern whether ApoE and related lipid proteins participated in the molecular network composed of proteins encoded by leprosy susceptibility genes, we constructed an interaction network of those genes (Zhang *et al.*[9] and references therein) using GeneMANIA.[38] We found that ApoE physically interacted, was co-expressed and genetically interacted with these proteins (such as C3, MRC1, MBL2, HLA and TAP1). Moreover, ApoE interacted with the lipid-related proteins (such as APP, APOB, APOC1, APOC3 and LIPC), which were also highly associated with the proteins encoded by the reported leprosy-risk genes (Fig. 2).

Discussion

Previous epidemiological studies have shown that elderly people with leprosy treated by antileprosy drugs had a significantly lower incidence of dementia compared with drug-free patients,[29] although there is some controversy.[44] Namba *et al.*[45] and Chui *et al.*[46] have found a significantly lower level of Aβ deposition in the brain tissues of elderly patients with leprosy than that of age-matched controls.

These studies suggested that patients with leprosy might have a very low risk of AD, and treatment with antileprosy drugs (e.g. dapsone, rifampicin and clofazimine) might play a role in reducing Aβ synthesis. However, there is not a consensus that these antileprosy drugs would have preventive effects on AD through inhibiting Aβ-induced neurotoxicity.[47–49] The molecular underpinning that would account for a low frequency of Aβ in brain tissues and a low incidence of dementia in patients with leprosy requires further study. The *APOE* gene is the well known susceptibility gene for sporadic late-onset AD.[26] It plays a critical role in the brain facilitating the proteolytic clearance of Aβ peptides and affects its homeostasis.[50] Therefore, we hypothesized that *APOE* would be involved in leprosy and we have provided evidence that this gene is associated with leprosy.

Among the five *APOE* SNPs analysed in this study, including three tag SNPs (rs405509, rs769450 and rs439401) and two AD-associated SNPs (rs429358 and rs7412, which define the *APOE* isoforms ε2, ε3 and ε4),[26] only SNPs rs405509 and rs439401 increased the risk of leprosy per se ($P < 0{\cdot}005$) and MB leprosy ($P < 0{\cdot}005$). These two SNPs were also reported to be associated with late-onset AD in a previous GWAS.[51] It should be noted that the significant association with leprosy per se appears to be caused by the skewing effect of patients with MB.

However, SNPs rs429358 and rs7412 showed no association with leprosy, and this negative result was confirmed by sequencing the *APOE* gene in the Wenshan sample (798 leprosy patients and 990 controls). Our results are different from a previous study that reported a significantly increased frequency of the *APOE*-ε4 allele in elderly patients with leprosy but not dementia.[52] Population differences might explain this.

We used the RegulomeDB database (www.regulomedb.org/index)[53] to annotate the analysed SNPs. All the five *APOE* SNPs showed a DNase hypersensitivity. Except for rs405509,

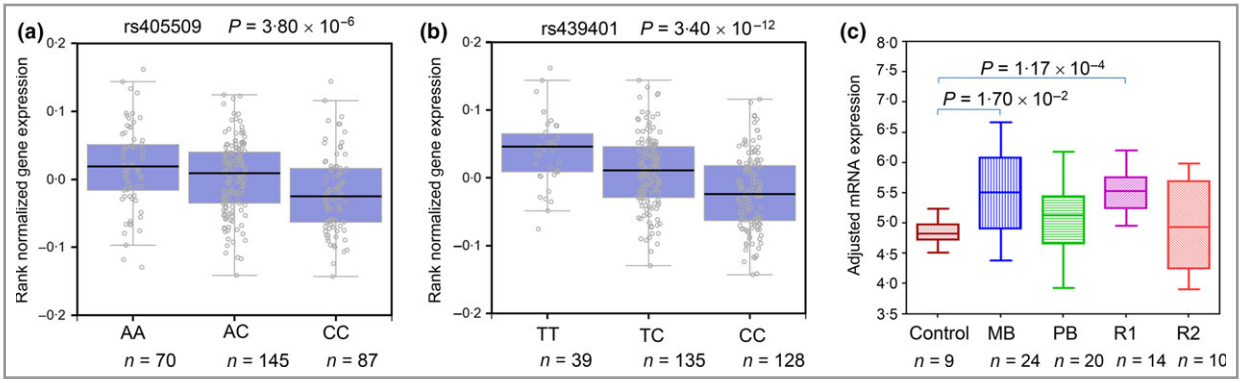

Fig 1. *APOE* gene expression analysis. Expression quantitative trait loci (eQTL) analysis of the *APOE* tag single-nucleotide polymorphisms in human skin tissues (a, b) using GTEx (http://www.gtexportal.org/home).[35] (c) Differential mRNA expression level of *APOE* in leprotic skin lesions was performed using microarray expression data GSE74481.[36] The dataset GSE74481 contains skin biopsies of 24 patients with multibacillary (MB) leprosy [10 mid-borderline leprosy (BB); 10 borderline lepromatous; and four lepromatous (LL)], 20 paucibacillary (PB) leprosy [10 tuberculoid (TT); 10 borderline-tuberculoid (BT)], 14 with type I reaction (R1) leprosy and 10 with type II reaction (R2), as well as normal skin biopsies from nine healthy individuals.

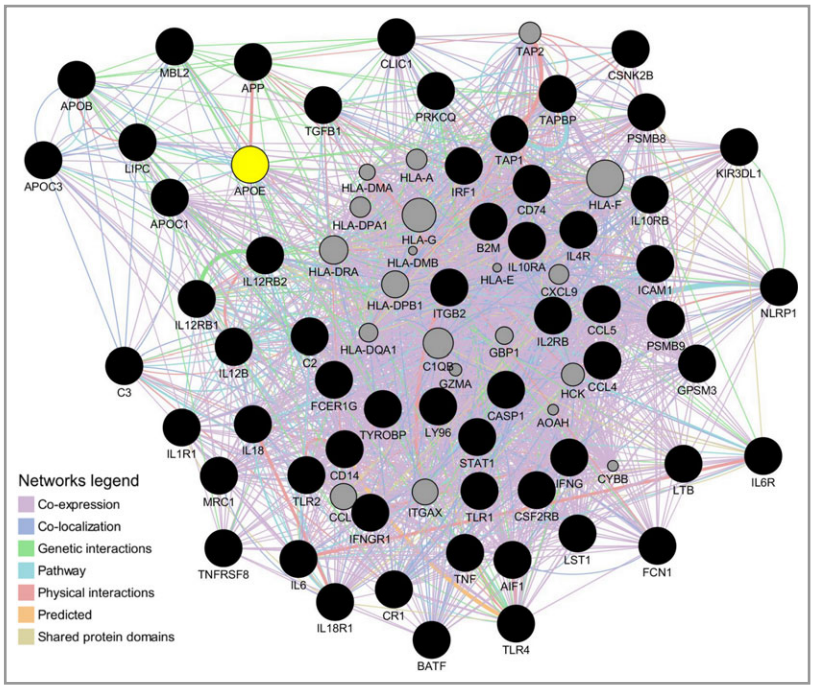

Fig 2. Interaction network of *APOE* and leprosy susceptibility genes. The network was composed of *APOE*, *APOE*-interactive lipid genes based on the literature and the reported leprosy susceptibility genes (Zhang *et al.*[9] and references therein) using the GeneMANIA prediction server (http://www.genemania.org/).[38] Hub nodes were defined by nodes with 40 connections or more in the interaction network.

rs769450 and rs429358, the other SNPs were located in transcription factor binding sites (Table S5; see Supporting Information). In order to consider the potential influence of genetic variation on gene expression,[54] we performed an eQTL analysis to elucidate whether these leprosy-risk genetic variants would alter *APOE* mRNA expression. We found that the two leprosy-risk SNPs rs405509 and rs439401 were *cis* eQTL for *APOE* expression in human skin, and the *APOE* mRNA had differential expression levels in skin lesions of R1 leprosy relative to control tissue.[36] We could not establish the association between genotypes of rs405509 and rs439401 and skin *APOE* mRNA expression, as the dataset reported by Belone and colleagues[36] lacked the related genetic information of *APOE* status. Nonetheless, the restricted eQTL effects of rs405509 and rs439401 in skin tissues (Fig. 1) were compatible with the differential *APOE* expression pattern in leprosy skin lesions.

During the invasion of *M. leprae* into human macrophage and Schwann cells, lipid transport, delivery and lipoprotein metabolism system genes play key roles.[55] *M. leprae* might utilize these genes to generate energy[31] and/or as a strategy for survival.[5,30] ApoE plays a central role in lipoprotein transportation and lipid metabolism.[56] Previous studies have shown that a higher level of ApoE expression in transgenic mice might significantly reduce plasma lipoproteins (e.g. cholesterol and triglycerides).[57,58] Concordantly, we found that the risk genotypes of both rs405509 and rs439401 had the lowest level of *APOE* expression (based on the eQTL data[35]), which would lead to increased plasma lipoprotein and facilitate the survival of *M. leprae*. This speculation was compatible with the observation that the skin lesions of patients with MB leprosy displayed a higher bacillary load than that of patients with PB leprosy.[59] Given the key roles of lipid proteins in *M. leprae* infection (e.g. as an energy source or immune response),[55] it is understandable that the *APOE* SNPs that were *cis* eQTL for *APOE* mRNA expression in human skin would affect susceptibility to MB. Combined with the observation that SNPs rs429358 and rs7412 (defining the *APOE* isoforms ε2, ε3 and ε4[26]) showed no association with leprosy, we speculated that the expression of *APOE*, but not its different isoforms, affected the onset of leprosy. The lack of association of SNPs rs429358 and rs7412 defining different *APOE* isoforms with leprosy would imply different roles of this protein in leprosy and AD.

It should be noted that the current sample size might be underpowered to detect the effect of SNPs rs429358 (MAF = 0·090) and rs7412 (MAF = 0·075). Nonetheless, the exact role and mechanism of ApoE in leprosy and AD await future study. Characterizing the role of ApoE in lipid metabolic processes during *M. leprae* infection and testing whether antileprosy drugs (e.g. rifampicin) affect ApoE expression would be a promising start.

In order to establish the potential role of ApoE in leprosy, we performed an interaction network analysis and found that *APOE*, together with other lipid-related genes, could interact with these proteins encoded by the previously reported leprosy susceptibility genes (Zhang *et al.*[9] and references therein). This observation provides an additional line of evidence that *APOE* might be involved in the molecular pathways composed of known leprosy susceptibility genes. For example, *APOE* was predicted to be connected with mannose binding lectin 2 (MBL2, which was reported to be associated with leprosy[18,19]) in the network (Fig. 2). This predicted connection was consistent with previous studies showing a putative interaction of ApoE and MBL2: the plasma ApoE and Mbl2 levels were correlated with diabetic phenotype in New Zealand obese mice[60] and *APOE* and *MBL* genotypes were associated with increased risk of neurocognitive impairment in

HIV-infected donors.[61] Similarly, we found a higher mRNA expression level of *MBL2* in skin lesions of patients with MB leprosy relative to control tissue (Table S6; see Supporting Information). Therefore, the information provided by network analysis might direct future functional experiments to elucidate its potential biological significance in leprosy.

In conclusion, we found that the *APOE* SNPs rs405509 and rs439401 were associated with leprosy, and the effect might be mediated by altered *APOE* mRNA expression and its potential role in lipid metabolic pathways during *M. leprae* infection. Future studies are needed to validate our observations and to explore the potential function of *APOE* during leprosy onset and progression.

References

1 Britton WJ, Lockwood DN. Leprosy. *Lancet* 2004; **363**:1209–19.
2 Leprosy as a neurological disease. *Lancet Neurol* 2009; **8**:217.
3 Ridley DS, Jopling WH. Classification of leprosy according to immunity. A five-group system. *Int J Lepr Other Mycobact Dis* 1966; **34**:255–73.
4 Scollard DM, Adams LB, Gillis TP *et al.* The continuing challenges of leprosy. *Clin Microbiol Rev* 2006; **19**:338–81.
5 Mattos KA, Lara FA, Oliveira VG *et al.* Modulation of lipid droplets by *Mycobacterium leprae* in Schwann cells: a putative mechanism for host lipid acquisition and bacterial survival in phagosomes. *Cell Microbiol* 2011; **13**:259–73.
6 World Health Organization. Chemotherapy of leprosy. Report of a WHO Study Group. *World Health Organ Tech Rep Ser* 1994; **847**:1–24.
7 World Health Organization. Global leprosy update, 2015: time for action, accountability and inclusion. *Wkly Epidemiol Rec* 2016; **91**:405–20.
8 Alter A, Grant A, Abel L *et al.* Leprosy as a genetic disease. *Mamm Genome* 2011; **22**:19–31.
9 Zhang D-F, Wang D, Li Y-Y *et al.* Integrative analyses of leprosy susceptibility genes indicate a common autoimmune profile. *J Dermatol Sci* 2016; **82**:18–27.
10 Siddiqui MR, Meisner S, Tosh K *et al.* A major susceptibility locus for leprosy in India maps to chromosome 10p13. *Nat Genet* 2001; **27**:439–41.
11 Mira MT, Alcais A, Van Thuc N *et al.* Chromosome 6q25 is linked to susceptibility to leprosy in a Vietnamese population. *Nat Genet* 2003; **33**:412–15.
12 Tosh K, Meisner S, Siddiqui MR *et al.* A region of chromosome 20 is linked to leprosy susceptibility in a South Indian population. *J Infect Dis* 2002; **186**:1190–3.
13 Zhang FR, Huang W, Chen SM *et al.* Genome-wide association study of leprosy. *N Engl J Med* 2009; **361**:2609–18.
14 Berrington WR, Macdonald M, Khadge S *et al.* Common polymorphisms in the *NOD2* gene region are associated with leprosy and its reactive states. *J Infect Dis* 2010; **201**:1422–35.
15 Sapkota BR, Macdonald M, Berrington WR *et al.* Association of TNF, MBL, and VDR polymorphisms with leprosy phenotypes. *Hum Immunol* 2010; **71**:992–8.
16 Alter A, de Leseleuc L, Van Thuc N *et al.* Genetic and functional analysis of common MRC1 exon 7 polymorphisms in leprosy susceptibility. *Hum Genet* 2010; **127**:337–48.
17 Wang D, Feng J-Q, Li Y-Y *et al.* Genetic variants of the MRC1 gene and the IFNG gene are associated with leprosy in Han Chinese from Southwest China. *Hum Genet* 2012; **131**:1251–60.
18 de Messias-Reason IJ, Boldt AB, Moraes Braga AC *et al.* The association between mannan-binding lectin gene polymorphism and clinical leprosy: new insight into an old paradigm. *J Infect Dis* 2007; **196**:1379–85.
19 Zhang D-F, Huang X-Q, Wang D *et al.* Genetic variants of complement genes ficolin-2, mannose-binding lectin and complement factor H are associated with leprosy in Han Chinese from Southwest China. *Hum Genet* 2013; **132**:629–40.
20 de Sales Marques C, Brito-de-Souza VN, Guerreiro LT *et al.* Toll-like receptor 1 N248S single-nucleotide polymorphism is associated with leprosy risk and regulates immune activation during mycobacterial infection. *J Infect Dis* 2013; **208**:120–9.
21 Shah JA, Berrington WR, Vary JC Jr *et al.* Genetic Variation in toll-interacting protein is associated with leprosy susceptibility and cutaneous expression of interleukin 1 receptor antagonist. *J Infect Dis* 2016; **213**:1189–97.
22 Wang D, Zhang DF, Feng JQ *et al.* Common variants in the PARL and PINK1 genes increase the risk to leprosy in Han Chinese from South China. *Sci Rep* 2016; **6**:37086.
23 Mahley RW, Rall SC Jr. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet* 2000; **1**:507–37.
24 Zhang CC, Ren HY, Li ML *et al.* Apolipoprotein E gene polymorphisms associated with processing speed and executive functions in healthy Han Chinese. *Neurosci Bull* 2015; **31**:368–70.
25 Raber J, Wong D, Buttini M *et al.* Isoform-specific effects of human apolipoprotein E on brain function revealed in *ApoE* knockout mice: increased susceptibility of females. *Proc Natl Acad Sci U S A* 1998; **95**:10914–19.
26 Corder EH, Saunders AM, Strittmatter WJ *et al.* Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993; **261**:921–3.
27 Sudlow C, Martinez Gonzalez NA, Kim J *et al.* Does apolipoprotein E genotype influence the risk of ischemic stroke, intracerebral hemorrhage, or subarachnoid hemorrhage? Systematic review and meta-analyses of 31 studies among 5961 cases and 17,965 controls. *Stroke* 2006; **37**:364–70.
28 Wilson PW, Schaefer EJ, Larson MG *et al.* Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. *Arterioscler Thromb Vasc Biol* 1996; **16**:1250–5.
29 McGeer PL, Harada N, Kimura H *et al.* Prevalence of dementia amongst elderly japanese with leprosy: apparent effect of chronic drug-therapy. *Dement Geriatr Cogn Disord* 1992; **3**:146–9.
30 Melo RC, Dvorak AM. Lipid body-phagosome interaction in macrophages during infectious diseases: host defense or pathogen survival strategy? *PLOS Pathog* 2012; **8**:e1002729.
31 Tanigawa K, Degang Y, Kawashima A *et al.* Essential role of hormone-sensitive lipase (HSL) in the maintenance of lipid storage in *Mycobacterium leprae*-infected macrophages. *Microb Pathog* 2012; **52**:285–91.
32 Li Y-Y, Li X-A, He L *et al.* Trends in new leprosy case detection over 57 years (1952–2008) in Yuxi, Yunnan Province of Southwest China. *Lepr Rev* 2011; **82**:6–16.
33 Chen XS, Li WZ, Jiang C *et al.* Leprosy in China: epidemiological trends between 1949 and 1998. *Bull World Health Organ* 2001; **79**:306–12.
34 International HapMap Consortium. The International HapMap Project. *Nature* 2003; **426**:789–96.
35 GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* 2013; **45**:580–5.
36 Belone Ade F, Rosa PS, Trombone AP *et al.* Genome-wide screening of mRNA expression in leprosy patients. *Front Genet* 2015; **6**:334.

37 Szklarczyk D, Franceschini A, Wyder S *et al.* STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res* 2015; **43**:D447–52.
38 Warde-Farley D, Donaldson SL, Comes O *et al.* The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res* 2010; **38**: W214–20.
39 Barrett JC, Fry B, Maller J *et al.* Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**:263–5.
40 Purcell S, Neale B, Todd-Brown K *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81**:559–75.
41 Gauderman WJ. Sample size requirements for matched case-control studies of gene-environment interaction. *Stat Med* 2002; **21**:35–50.
42 Lek M, Karczewski KJ, Minikel EV *et al.* Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016; **536**:285–91.
43 1000 Genomes Project Consortium, Auton A, Brooks LD *et al.* A global reference for human genetic variation. *Nature* 2015; **526**:68–74.
44 Goto M, Kimura T, Hagio S *et al.* Neuropathological analysis of dementia in a Japanese leprosarium. *Dementia* 1995; **6**:157–61.
45 Namba Y, Kawatsu K, Izumi S *et al.* Neurofibrillary tangles and senile plaques in brain of elderly leprosy patients. *Lancet* 1992; **340**:978.
46 Chui DH, Tabira T, Izumi S *et al.* Decreased beta-amyloid and increased abnormal Tau deposition in the brain of aged patients with leprosy. *Am J Pathol* 1994; **145**:771–5.
47 Tomiyama T, Asano S, Suwa Y *et al.* Rifampicin prevents the aggregation and neurotoxicity of amyloid beta protein in vitro. *Biochem Biophys Res Commun* 1994; **204**:76–83.
48 Umeda T, Ono K, Sakai A *et al.* Rifampicin is a candidate preventive medicine against amyloid-beta and tau oligomers. *Brain* 2016; **139**:1568–86.
49 Endoh M, Kunishita T, Tabira T. No effect of anti-leprosy drugs in the prevention of Alzheimer's disease and beta-amyloid neurotoxicity. *J Neurol Sci* 1999; **165**:28–30.
50 Jiang Q, Lee CY, Mandrekar S *et al.* ApoE promotes the proteolytic degradation of Abeta. *Neuron* 2008; **58**:681–93.
51 Abraham R, Moskvina V, Sims R *et al.* A genome-wide association study for late-onset Alzheimer's disease using DNA pooling. *BMC Med Genomics* 2008; **1**:44.
52 Endoh M, Ueki A, Takahashi K *et al.* Significantly increased frequency of the apolipoprotein E epsilon 4 allele in elderly non-demented leprosy patients. *Neurosci Lett* 1996; **207**:206–8.
53 Boyle AP, Hong EL, Hariharan M *et al.* Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res* 2012; **22**:1790–7.
54 Francesconi M, Lehner B. The effects of genetic variation on gene expression dynamics during development. *Nature* 2014; **505**:208–11.
55 Elamin AA, Stehr M, Singh M. Lipid droplets and *Mycobacterium leprae* infection. *J Pathog* 2012; **2012**:361–74.
56 Huang Y, Mahley RW. Apolipoprotein E: structure and function in lipid metabolism, neurobiology, and Alzheimer's diseases. *Neurobiol Dis* 2014; 7**2 Pt A**:3–12.
57 Shimano H, Yamada N, Katsuki M *et al.* Overexpression of apolipoprotein E in transgenic mice: marked reduction in plasma lipoproteins except high density lipoprotein and resistance against diet-induced hypercholesterolemia. *Proc Natl Acad Sci U S A* 1992; **89**:1750–4.
58 Hasty AH, Linton MF, Swift LL *et al.* Determination of the lower threshold of apolipoprotein E resulting in remnant lipoprotein clearance. *J Lipid Res* 1999; **40**:1529–38.
59 Walker SL, Lockwood DN. The clinical and immunological features of leprosy. *Br Med Bull* 2006; **77–78**:103–21.
60 von Toerne C, Kahle M, Schafer A *et al.* Apoe, Mbl2, and Psp plasma protein levels correlate with diabetic phenotype in NZO mice–an optimized rapid workflow for SRM-based quantification. *J Proteome Res* 2013; **12**:1331–43.
61 Spector SA, Singh KK, Gupta S *et al.* APOE epsilon4 and MBL-2 O/O genotypes are associated with neurocognitive impairment in HIV-infected plasma donors. *AIDS* 2010; **24**:1471–9.
62 Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Res* 2003; **31**:3812–14.
63 Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 2009; **4**:1073–81.
64 Adzhubei IA, Schmidt S, Peshkin L *et al.* A method and server for predicting damaging missense mutations. *Nat Methods* 2010; **7**:248–9.
65 Chun S, Fay JC. Identification of deleterious mutations within three human genomes. *Genome Res* 2009; **19**:1553–61.
66 Schwarz JM, Cooper DN, Schuelke M *et al.* MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods* 2014; **11**:361–2.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1 Primers for genotyping of the *APOE* gene.

Table S2 Genotype and allele frequencies of five single-nucleotide polymorphisms in 527 patients with leprosy and 583 healthy controls from Yuxi, Yunnan Province, China (adjusted by sex).

Table S3 Distribution of the *APOE* haplotypes in 527 patients with leprosy and 583 healthy controls from Yuxi, Yunnan Province, China.

Table S4 List of rare single-nucleotide polymorphisms in the exon and flanking regions in the *APOE* gene in 798 patients with leprosy and 990 controls using next-generation sequencing technologies.

Table S5 Single-nucleotide polymorphism annotation with known and predicted regulatory elements.

Table S6 mRNA expression levels of the *MBL2* gene in leprosy skin lesions.

Fig S1. The linkage disequilibrium structure of *APOE* in patients with leprosy and healthy controls from the Yuxi prefecture, Yunnan Province, China.

Fig S2. Statistical power estimates for the case–control association analysis.

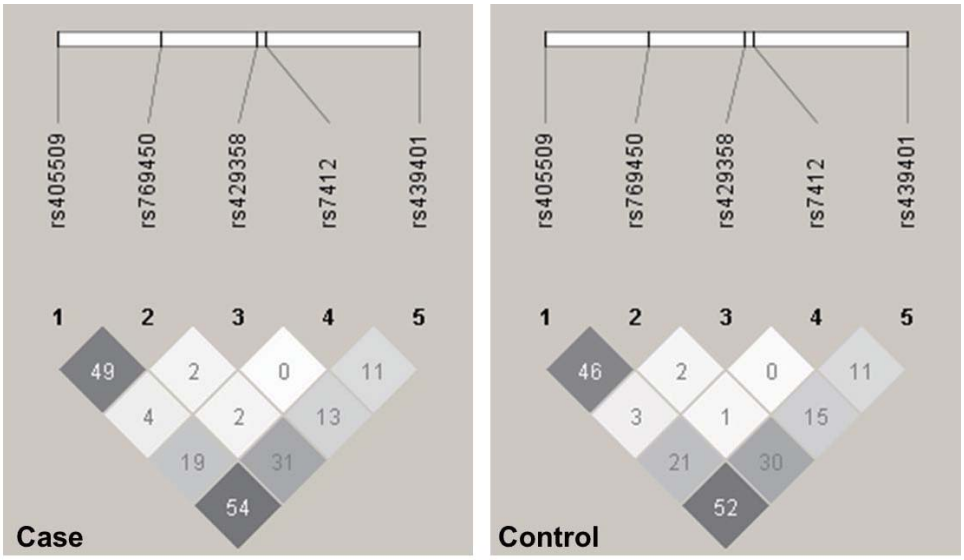

Figure S1. The linkage disequilibrium (LD) structure of *APOE* in leprosy patients and healthy controls from the Yuxi Prefecture, Yunnan, China. Black squares represent high LD as measured by r^2, gradually coloring down to white squares of low LD. The individual square showed the r^2 value for each SNP pair (r^2 value is multiplied by 100).

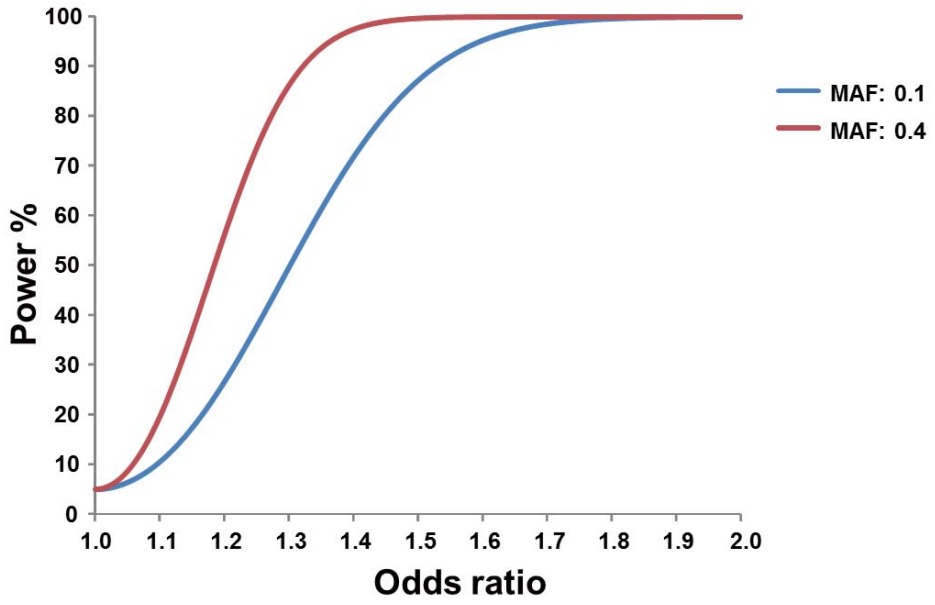

Figure S2. Statistical power estimates for the case-control association analysis. The following parameters were used: gene only hypothesis; the log additive model; case, n = 527; control, n= 583; risk allele frequency = 0.1/0.4; range of OR from 1.0 to 2.0; two-sided type I error rate = 0.05.

Table S1. Primers for genotyping of the *APOE* gene

SNP	Location	Primer (5'-3')
For SNaPshot:		
rs405509	tagSNPs, 5'UTR	Forward: ATAGAGGTCTTTTGACCACCC
		Reverse: ATTCCCCTTCCACGCTTG
		Probe: ct$(gact)_8$CTGACTGACTGACTGACTGACTGACTGACT
rs769450	tagSNPs, Intron	Forward: TTTCGATCTCCCAAAGTGC
		Reverse: TGTCTGGTATTCACTATCTGCCT
		Probe:
rs439401 [a]	tagSNPs, 3' region	Forward: AGGGGAAGCTTGGATGGA
		Reverse: TTGGTGGGAACCCTCCTC
		Probe: ct$(gact)_8$CTGACTGACTGACTGACTGACTGACTGACT
For PCR and Sequencing:		
rs429358 and rs7412	exon	Forward: CGGAACTGGAGGAACAACTGA
		Reverse: GCCCCGGCCTGGTACACT

(GACT)n, n repeats of "GACT"

[a] rs439401 was not genotyped in the same SNaPshot panel for rs405509 and rs769450.

Table S2 Genotype and allele frequencies of 5 SNPs in 527 leprosy patients and 583 healthy controls from Yuxi, Yunnan Province, China (adjusted by sex)

		Cases versus controls		MB versus controls		PB versus controls	
	Genotype/Allele	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)
rs405509	AA	reference		reference		reference	
C/A	CA	**0.003**	1.467(1.143-1.883)	**0.006**	1.522(1.126-2.059)	0.042	1.386(1.012-1.896)
	CC	0.405	1.212(0.771-1.905)	0.348	1.298(0.753-2.235)	0.644	1.146(0.643-2.043)
	A allele	reference		reference		reference	
	C allele	0.025	1.235(1.027-1.485)	0.027	1.283(1.029-1.600)	0.135	1.193(0.946-1.504)
	CC+CA	**0.005**	1.412(1.112-1.793)	**0.007**	1.484(1.111-1.981)	0.054	1.345(0.995-1.817)
rs769450	GG	reference		reference		reference	
A/G	AG	0.993	0.997(0.549-1.812)	0.910	1.043(0.502-2.169)	0.966	0.983(0.461-2.096)
	AA	0.012	1.396(1.075-1.813)	0.014	1.475(1.080-2.013)	0.151	1.272(0.916-1.767)
	G allele	reference		reference		reference	
	A allele	0.076	1.211(0.980-1.498)	0.060	1.273(0.989-1.639)	0.308	1.149(0.880-1.501)
	AA+AG	0.026	1.328(1.034-1.705)	0.023	1.415(1.050-1.908)	0.195	1.232(0.899-1.687)
rs429358	TT	reference		reference		reference	
C/T	CT	0.960	1.036(0.256-4.192)	0.989	0.988(0.179-5.469)	0.893	1.125(0.203-6.239)
	CC	0.232	1.206(0.887-1.638)	0.545	1.122(0.773-1.628)	0.237	1.255(0.861-1.828)
	T allele	reference		reference		reference	
	C allele	0.270	1.171(0.884-1.550)	0.579	1.101(0.783-1.549)	0.266	1.215(0.862-1.712)
	CC+CT	0.273	1.183(0.876-1.599)	0.556	1.116(0.774-1.611)	0.239	1.250(0.863-1.810)
rs7412	CC	reference		reference		reference	
T/C	TC	0.317	1.181(0.853-1.637)	0.468	1.157(0.781-1.714)	0.311	1.232(0.823-1.846)
	TT	-	-	-	-	-	-
	C allele	reference		reference		reference	
	T allele	0.419	1.137(0.833-1.550)	0.566	1.116(0.767-1.623)	0.402	1.178(0.803-1.730)
	TT+TC	0.354	1.166(0.842-1.615)	0.508	1.142(0.771-1.691)	0.342	1.216(0.812-1.820)
rs439401	TT	reference		reference		reference	
C/T	CT	0.085	1.366(0.958-1.948)	0.264	1.286(0.827-2.002)	0.113	1.427(0.919-2.214)
	CC	**0.0005**	1.611(1.234-2.105)	**0.001**	1.741(1.257-2.411)	0.024	1.476(1.052-2.072)
	T allele	reference		reference		reference	
	C allele	0.017	1.231(1.038-1.460)	0.055	1.224(0.996-1.503)	0.049	1.240(1.001-1.537)
	CC+CT	**0.001**	1.532(1.190-1.972)	**0.002**	1.616(1.185-2.205)	0.020	1.463(1.062-2.014)

MB, multibacillary leprosy; PB, paucibacillary leprosy; OR, odds ratio; 95% CI, 95% confidence interval
*All data were calculated by using the unconditional logistic regression, with an adjustment for sex. The major genotype or allele was served as reference. *P*-values less than 0.01 (Bonferroni correction: 0.05/5) were marked in bold.

Table S3. Distribution of the *APOE* haplotypes in 527 leprosy cases and 583 healthy controls from Yuxi, Yunnan, China

Haplotype	Control	Case vs. Control		MB vs. Control		PB vs. Control	
	Freq.	Freq.	*P* *	Freq.	*P* *	Freq.	*P* *
rs405509-rs769450-rs429358-rs7412-rs439401							
A-G-T-C-T	0.611	0.563	0.023	0.565	0.068	0.561	0.060
C-A-T-C-C	0.164	0.200	0.030	0.207	0.029	0.191	0.188
A-G-C-C-C	0.090	0.098	0.515	0.095	0.728	0.101	0.466
C-G-T-T-C	0.074	0.083	0.435	0.081	0.575	0.084	0.458
C-G-T-C-C	0.026	0.025	0.874	0.026	0.996	0.023	0.788
A-G-T-C-C	0.023	0.023	0.989	0.019	0.539	0.029	0.522
A-A-T-C-C	0.013	0.009	0.392	0.007	0.340	0.011	0.704
rs405509-rs439401							
AT	0.610	0.557	**0.012**	0.560	0.047	0.554	0.034
CC	0.265	0.313	**0.015**	0.319	0.022	0.306	0.096
AC	0.125	0.130	0.677	0.121	0.856	0.141	0.374
rs429358-rs7412							
ε2 (TT)	0.074	0.084	0.396	0.083	0.555	0.084	0.481
ε3 (TC)	0.833	0.810	0.168	0.817	0.431	0.807	0.177
ε4 (CC)	0.093	0.106	0.317	0.101	0.633	0.110	0.273

MB – multibacillary leprosy; PB – paucibacillary leprosy; Freq - frequency; *P* - *P* value

* Bonferroni correction for multiple comparisons was used.

ε2 – *APOE* type 2; ε3 – *APOE* type 3; ε4 – *APOE* type 4

Table S4. The list of rare SNPs in the exon and flank regions in the *APOE* gene in 798 leprosy cases and 990 controls by using the next-generation sequencing technologies

Position	SNP ID	Function	Ref.	Alt.	Residue change	Damaging predication [a]	Allele counts in leprosy patients	Allele counts in healthy controls	*P*-value [b]	OR [c]	Allele counts in ExAC-EAS	*P*-value [b]	OR [c]
chr19:45409053	.	utr-5	C	T	-	-	2/1594	0/1978	0.199	6.212	NA	-	-
chr19:45409136	rs373985746	intron	G	A	-	-	8/1590	11/1974	1.000	0.902	1/480	0.694	2.422
chr19:45409844	.	intron	T	C	-	-	1/1596	0/1980	0.446	3.724	NA	-	-
chr19:45409948	rs373651604	intron	T	C	-	-	3/1596	0/1980	0.089	8.700	5/8652	0.115	3.257
chr19:45409950	rs530431608	intron	C	T	-	-	2/1596	1/1980	0.589	2.483	NA	-	-
chr19:45411025	rs533904656	missense	G	A	p.A18T	Tolerated	12/1594	2/1980	0.002	7.502	13/6606	0.001	3.847
chr19:45411072	.	synonymous	C	T	p.R33	-	1/1596	1/1978	1.000	1.240	NA	-	-
chr19:45411082	rs142480126	missense	G	A	p.E37K	Tolerated	1/1596	0/1980	0.446	3.724	0/8504	-	-
chr19:45411089	.	missense	A	G	p.Q39R	Tolerated	1/1596	1/1980	1.000	1.241	3/8534	0.496	1.783
chr19:45411153	.	missense	A	C	p.T60S	-	0/1596	1/1974	1.000	0.412	NA	-	-
chr19:45411190	.	missense	C	A	p.Q73K	Tolerated	0/1596	1/1974	1.000	0.412	NA	-	-
chr19:45411762	rs372675300	intron	C	T	-	-	3/1596	9/1978	0.246	0.412	18/8502	1.000	0.888
chr19:45411876	.	missense	G	A	p.R108Q	Tolerated	1/1596	2/1980	1.000	0.620	1/5338	0.407	3.346
chr19:45411944	.	missense	G	A	p.G131N	Tolerated	2/1596	0/1980	0.199	6.210	NA	-	-
chr19:45411945	.	missense	G	A	p.G131N	Tolerated	1/1596	5/1980	0.235	0.248	NA	-	-
chr19:45411950	.	synonymous	C	T	p.L133L	-	1/1596	2/1980	1.000	0.620	NA	-	-
chr19:45411965	rs543363163	missense	G	A	p.G138S	Tolerated	2/1596	1/1978	0.590	2.481	0/562	-	-
chr19:45411977	.	missense	G	A	p.A142N	Tolerated	1/1596	0/1974	0.447	3.713	NA	-	-
chr19:45411978	.	missense	C	A	p.A142N	Damaging	1/1596	1/1974	1.000	1.237	NA	-	-
chr19:45411983	.	missense	C	T	p.L144F	Damaging	1/1596	5/1974	0.234	0.247	NA	-	-
chr19:45412060	.	synonymous	T	C	p.D169	-	1/1592	1/1966	1.000	1.235	NA	-	-
chr19:45412061	.	missense	G	A	p.A170T	Tolerated	0/1592	2/1966	0.505	0.247	NA	-	-
chr19:45412066	.	missense	T	G	p.D171E	Tolerated	1/1590	1/1966	1.000	1.237	NA	-	-

chr19:45412087	.	synonymous	A	C	p.A178	-	0/1590	1/1958	1.000	0.410	NA	-	-
chr19:45412099	.	synonymous	C	G	p.A182	-	0/1592	2/1956	0.505	0.245	NA	-	-
chr19:45412103	.	missense	G	C	p.A184L	Damaging	1/1592	1/1952	1.000	1.226	NA	-	-
chr19:45412104	.	missense	C	T	p.A184L	Tolerated	2/1590	1/1948	0.591	2.452	NA	-	-
chr19:45412105	.	missense	C	G	p.A184L	Tolerated	0/1588	1/1948	1.000	0.409	NA	-	-
chr19:45412124	.	missense	G	A	p.G191S	Tolerated	0/1578	2/1944	0.505	0.246	NA	-	-
chr19:45412127	.	missense	C	G	p.L192V	Tolerated	1/1576	2/1942	1.000	0.616	NA	-	-
chr19:45412129	.	missense	C	G	p.L192V	Tolerated	0/1576	2/1944	0.505	0.246	NA	-	-
chr19:45412136	.	missense	A	C	p.I195L	Tolerated	0/1578	2/1944	0.505	0.246	NA	-	-
chr19:45412165	.	synonymous	A	G	p.E204E	-	1/1584	0/1938	0.450	3.673	NA	-	-
chr19:45412175	.	missense	G	T	p.V208L	Tolerated	1/1590	1/1940	1.000	1.220	NA	-	-
chr19:45412190	.	missense	G	C	p.V213L	Tolerated	1/1592	0/1954	0.449	3.684	NA	-	-
chr19:45412193	.	missense	G	A	p.G214S	Tolerated	1/1592	1/1954	1.000	1.228	0/68	-	-
chr19:45412199	.	missense	C	A	p.L216R	Tolerated	1/1594	1/1958	1.000	1.229	NA	-	-
chr19:45412218	.	missense	A	G	p.Q222R	Tolerated	0/1592	1/1954	1.000	0.409	NA	-	-
chr19:45412219	.	missense	G	C	p.Q222R	Tolerated	0/1592	1/1956	1.000	0.409	NA	-	-
chr19:45412231	.	missense	G	A	p.Q226E	Tolerated	0/1592	1/1960	1.000	0.410	NA	-	-
chr19:45412245	.	missense	G	A	p.R231K	Tolerated	1/1590	0/1966	0.447	3.712	NA	-	-
chr19:45412254	.	missense	C	G	p.A234G	Tolerated	0/1592	1/1970	1.000	0.412	NA	-	-
chr19:45412255	.	missense	G	A	p.A234G	Tolerated	1/1594	3/1970	0.633	0.412	NA	-	-
chr19:45412259	.	missense	A	C	p.M236L	Tolerated	1/1594	1/1970	1.000	1.236	NA	-	-
chr19:45412297	.	synonymous	C	T	p.D248D	-	3/1596	0/1974	0.089	8.674	NA	-	-
chr19:45412301	.	missense	G	A	p.V250M	Tolerated	1/1596	0/1974	0.447	3.713	NA	-	-
chr19:45412305	.	missense	A	G	p.K251R	Tolerated	0/1596	1/1974	1.000	0.412	NA	-	-
chr19:45412306	.	missense	G	T	p.K251R	Tolerated	0/1596	1/1974	1.000	0.412	NA	-	-
chr19:45412313	.	missense	G	C	p.V254L	Tolerated	1/1596	0/1974	0.447	3.713	NA	-	-
chr19:45412317	.	missense	C	A	p.A255E	Tolerated	0/1596	1/1972	1.000	0.412	NA	-	-
chr19:45412328	.	missense	G	A	p.A259T	Tolerated	1/1596	4/1976	0.389	0.309	NA	-	-
chr19:45412334	.	missense	C	G	p.L261V	Tolerated	0/1596	2/1978	0.506	0.248	NA	-	-
chr19:45412349	.	missense	C	A	p.Q266R	Tolerated	0/1596	2/1978	0.506	0.248	NA	-	-

chr19:45412350	.	missense	A	G	p.Q266R	Tolerated	0/1596	1/1978	1.000	0.413	NA	-	-
chr19:45412357	.	missense	A	G	p.I268L	Tolerated	0/1596	1/1978	1.000	0.413	NA	-	-
chr19:45412393	.	synonymous	G	A	p.K280	-	0/1596	2/1980	0.506	0.248	NA	-	-
chr19:45412394	.	missense	A	G	p.S281G	Tolerated	1/1596	0/1980	0.446	3.724	NA	-	-
chr19:45412425	.	missense	A	G	p.Q291R	Tolerated	1/1596	0/1978	0.447	3.720	NA	-	-
chr19:45412537	.	utr-3	C	G	-	-	2/1592	1/1966	0.590	2.472	NA	-	-

The variant with a frequency <1% in either patient or control group was regarded as rare variant. Chr, Chromosome; Ref, Reference allele; Alt, Alternate allele; ExAC-EAS, 4,327 East Asian (EAS) individuals in the ExAC database [1]; OR, Odds ratio; NA, no data available.

[a] Missense variants are rated as damaging when at least two of five prediction algorithms (SIFT [2,3], PolyPhen2 HumDiv, PolyPhen2 HumVar [4], LRT [5] and MutationTaster [6]) suggesting a potential deleterious effect, otherwise the variants are rated as tolerated.

[b] *P*-values were calculated by using the Fisher's exact test. All *P*-values were not statistically significant after Bonferroni correction.

[c] The Odds ratio (OR) for the rare variant should be received with caution, as it was biased due to its absence /extremely low frequency in either patient or control group.

Table S5. SNP annotation with known and predicted regulatory elements

SNP	Annotation
rs405509	eQTL + DNase peak
rs769450	DNase peak
rs429358	DNase peak
rs7412	TF binding + DNase peak
rs439401	eQTL + TF binding + any motif + DNase Footprint + DNase peak
rs373985746	TF binding + DNase peak
rs440446	TF binding + DNase peak

DNase peak: DNase sensitivity; TF binding: transcription factor binding sites
Note: The RegulomeDB dataset (http://www.regulomedb.org/index) [7] includes public datasets from GEO, the ENCODE project, and published literature.

Table S6. mRNA expression levels of the *MBL2* gene in leprosy skin lesion

	t	B	logFC	*P*. Val	adj. *P*. Val
Control vs. MB	5.52	3.86	0.89	3.55×10^{-6}	2.42×10^{-5}
Control vs. PB	-6.48	6.20	-1.15	3.59×10^{-7}	3.90×10^{-6}
Control vs. R1	7.79	7.94	0.91	4.89×10^{-8}	2.72×10^{-7}
Control vs. R2	-5.74	2.68	-1.57	1.51×10^{-5}	7.16×10^{-5}

t, Moderated *t*-statistic; B, B-statistic or log-odds that the gene is differentially expressed; logFC, Log2 fold change between two experimental conditions; *P*. Val , raw *P*-value; adj. *P*. Val, *P* value after adjustment for multiple testing.

Note: The microarray expression data were retrieved from GEO according to accession series GSE74481 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74481)[8]. This dataset contains skin biopsies of 24 MB (10 mid-borderline leprosy [BB] + 10 borderline lepromatous [BL] + 4 lepromatous [LL]), 20 PB (10 tuberculoid [TT] + 10 borderline-tuberculoid [BT]), 14 type I reaction (R1), 10 type II reaction (R2) patients, and 9 healthy individuals.