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The mtDNA replication-related genes *TFAM* and *POLG* are associated with leprosy in Han Chinese from Southwest China

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ABSTRACT

Background: The pathogen *Mycobacterium leprae* of leprosy is heavily dependent on the host energy metabolites and nutritional products for survival. Previously we and others have identified associations of several mitochondrion-related genes and mitochondrial DNA (mtDNA) copy number alterations with leprosy and/or its subtype. We hypothesized that genetic variants of mtDNA replication-related genes would affect leprosy.

Objective: We aimed to identify genetic associations between the mtDNA replication-related genes *TFAM, POLG* and leprosy.

Methods: Genetic association study was performed in 2898 individuals from two independent sample sets in Yunnan Province, China. We first screened 7 tag SNPs of *TFAM* and *POLG* in 527 leprosy cases and 583 controls (Sample I). Expression quantitative trait loci (eQTL) analysis and differential mRNA expression were analyzed to discern potential effect of risk variants. The entire exon region of *TFAM* and *POLG* were further analyzed in 798 leprosy cases and 990 controls (Sample II; 4327 East Asians from the ExAC dataset was included as a reference control) by using targeted gene sequencing for fine mapping potentially causal variants.

Results: Two tag SNPs of *TFAM* (rs1049432, $P = 0.007$) and *POLG* (rs3176238, $P = 0.006$) were associated with multibacillary leprosy (MB) in Sample I and the significance survived correction for multiple comparisons. SNPs rs1937 of *TFAM* (which was linked with rs1049432) and rs61756401 of *POLG* were associated with leprosy, whereas no potentially causative coding variants were identified in Sample II. The eQTL analysis showed that rs1049432 was a significant *cis* eQTL for *TFAM* in nerve tissue ($P = 1.20 \times 10^{-12}$), and rs3176238 was a significant *cis* eQTL for *POLG* in nerve ($P = 3.90 \times 10^{-13}$) and skin tissues ($P = 2.50 \times 10^{-11}$). Consistently, mRNA level of *POLG* was differentially expressed in leprotic skin lesions.

Conclusions: Genetic variants of *TFAM* and *POLG* were associated with leprosy in Han Chinese, presumably by affecting gene expression.

1. Introduction

Leprosy is a chronic infectious and neurological disease [1,2] that affects around 200,000 people each year [3]. The pathogen, *Mycobacterium leprae* (*M. leprae*), is an obligate intracellular parasite with an extremely eroded genome [4,5]. Nearly 50% of coding genes in *M. leprae* genome, especially these genes in energy metabolic pathways, were pseudogenized or lost [4,5]. This evolutionary event led to an essential dependence of the host energy metabolites and nutritional products for the survival of *M.*

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leprae.

Mitochondria play key roles in cellular energy supply and antimicrobial immune response [6,7] and may be actively involved in the infection of *M. leprae* given its eroded genome and dependence on host metabolism [8]. An increasing number of studies showed that mitochondrial dysfunction could cause heart, nervous and immune system diseases [6,7,9]. Recently, we found that the mitochondrion-related genes that were encoded by chromosomal genes, such as *OPA1* [10], *LRRK2* [11], *PINK1* and *PARL* [12] were associated with leprosy *per se* and/or clinical subtypes, but the mitochondrion-related antimicrobial/antiviral immune genes *MAVS*, *MITA* and *MFN2* were not associated with leprosy [13]. In patients with lepromatous leprosy (LL), there was a higher mitochondrial DNA (mtDNA) copy number than those with the other clinical subtypes [8], which might be caused by the alteration of the host mtDNA replication effect or autophagy [14] after the load of *M. leprae* and different host immune reactions. In this study, we hypothesized that mtDNA replication-related genes *mitochondrial transcription factor A* (*TFAM*) and *mtDNA polymerase gamma* (*POLG*) were associated with leprosy and this might explain the altered mtDNA copy number in lepromatous leprosy [8]. TFAM is an essential protein component of the mitochondrial nucleoid core complex [15]. It plays a key role in mtDNA transcription, replication and repair [16,17]. Previous association study showed that rs2306604 of *TFAM* affects the risk for Alzheimer's disease (AD) [18], and mtDNA copy number was increased in *TFAM* transgenic animals [19]. The *POLG* gene is located in chromosome 15q26 and encodes a catalytic subunit of mtDNA polymerase [20]. It also plays an essential role in mtDNA replication and repair [21]. Dysfunction of POLG has been reported in Parkinson's disease (PD) [22,23] and other mitochondrial syndromes [24]. Leprosy was regarded as a neurological disease [2] and it would be naturally to test potential associations of *TFAM* and *POLG* with leprosy given the roles of these two genes in neurological diseases [18,22,23].

In this study, we performed a genetic association study to discern potential association of *TFAM* and *POLG* with leprosy in two independent sample sets from Yunnan Province, China. Seven tag single nucleotide polymorphisms (SNPs) of *TFAM* and *POLG* were analyzed in Sample I (527 leprosy cases and 583 controls). Together with expression quantitative trait loci (eQTL) analysis and differential mRNA expression analysis of leprotic skin lesions, we aimed to characterize the roles of *TFAM* and *POLG* in leprosy. We also directly sequenced the complete coding region of *TFAM and POLG* in Sample II (798 leprosy cases and 990 controls) using the next-generation sequencing (NGS) technologies to validate the association and to identify potential causal variants. Our results indicated that *TFAM* and *POLG* confer genetic susceptibility to leprosy in Han Chinese.

2. Materials and methods

2.1. Subjects

A total of 2898 individuals were collected from the Yuxi Prefecture and Wenshan Prefecture, Yunnan Province of Southwest China. The Yuxi sample served as Sample I and had been described in our previous studies [10,11]. In brief, 527 leprosy patients (onset age from 2 to 67 years, 387 males and 140 females, 279 multibacillary [MB] and 248 paucibacillary [PB] patients) and 583 healthy control subjects (age from 4 to 88 years, 365 males and 218 females) were collected from the same geographic region. The Wenshan sample was used as Sample II and contained 798 leprosy patients (onset age from 4 to 87 years, mean age: 26.7 ± 12.7 years; male/female ratio = 547/251; 452 MB and 311 PB patients, excluding 35 patients with unclear information for PB and MB assignment) and 990 healthy controls (age from 9 to 83 years, mean age: 38.1 ± 14.0 years; male/female ratio = 551/439) from the same geographic region. All patients were diagnosed by clinical and histopathological features and/or bacteriological index (if available), as had been described in our previous epidemiological study for leprosy [25]. The regionally-matched healthy individuals had no history of leprosy, HIV infection, and tuberculosis. Written informed consents 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 the experimental protocols of this study.

2.2. SNP selection

The tag SNPs of *TFAM* and *POLG* were selected according to the linkage disequilibrium (LD, $r^2 \geq 0.8$, minor allele frequency (MAF) ≥ 0.2) pattern in the HapMap Han Chinese in Bejing (CHB) population [26] (Fig. 1), following the same strategy and rationale as described in our previous studies [27]. In brief, four tag SNPs (rs10826175, rs11006127, rs10826179, and rs1049432) were chosen, with a capability to capture more than 90% (25/27) of all common SNPs (MAF ≥ 0.2) within *TFAM*. Three tag SNPs (rs1138465, rs3176238 and rs2247233) were chosen for *POLG*,

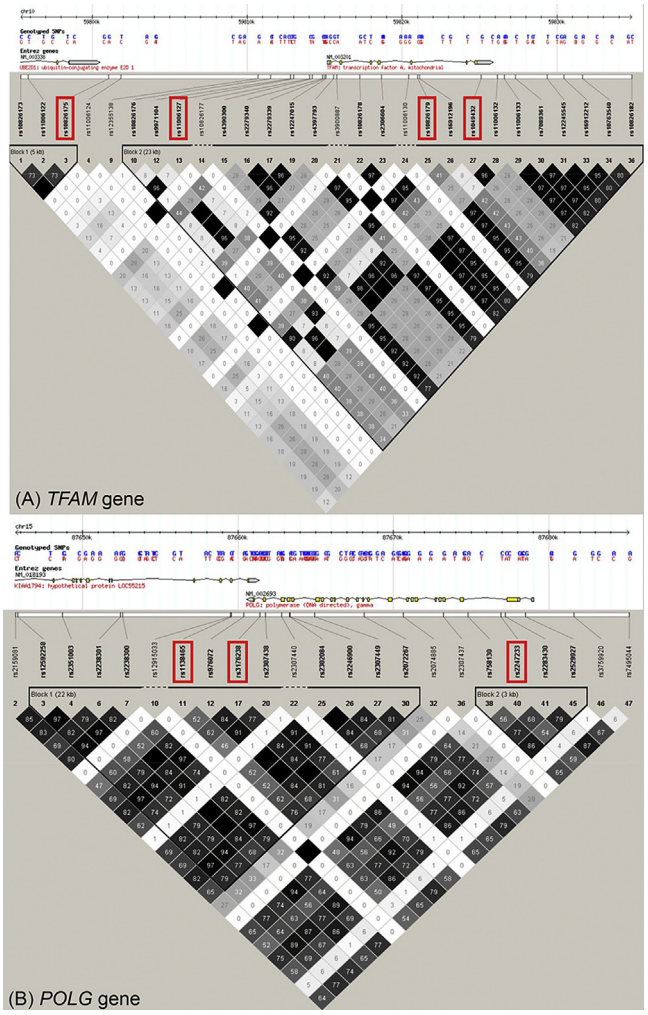

Fig. 1. Linkage disequilibrium plot of the *TFAM* SNPs (A) and *POLG* SNPs (B) based on the CHB dataset of HapMap, Phase 3 [26]. Only these SNPs with a MAF > 0.2 were considered and the selected SNPs for genotyping were marked in red box.

capturing 87% of all common SNPs (28/32) with a MAF ≥ 0.2 within this gene.

2.3. Genotyping and targeted gene sequencing

The 7 tag SNPs were genotyped in Sample I using the SNaPshot assay following the procedure described in our previous studies [10,12,27]. The primers used for genotyping were listed in Table S1.

We sequenced Sample II by using the NimbleGene SeqCap EZ Human Exome v3.0 (Roche) on the Illumina Hiseq 4000 platform at the Kunming Biological Diversity Regional Center of Instruments. The alignment and variant calling for the exons were performed by the same procedure in our previous studies [12,13]. Moreover, exome data of 4327 East Asians (EAS, containing CHB, CHS, Chinese Dai – Xishuangbanna (CDX), Japanese in Tokyo (JPT) and Kinh in Ho Chi Minh City, Vietnam (KHV)) from the Exome Aggregation Consortium (ExAC) dataset (http://exac.broadinstitute.org/; [28]) were retrieved as another control sample for comparison. In details, we downloaded a local copy of the ExAC dataset (release 0.3.1), a VCF file that contains variant annotation and its frequency in global populations. We retrieved the studied gene and variants from the EAS populations using an in-house Perl script. Allele frequency differences between the case and control groups were calculated by the Fisher's exact test.

Due to different genotyping platforms used in this study for the two sample sets, none of the 7 tag SNPs analyzed in Sample I were located in the exon and flanking regions that could be captured by the targeted gene sequencing in Sample II. On this point, Sample I was used for initial screening to discern potential association of the candidate genes with leprosy and Sample II was used for fine-mapping of causal variants in the leprosy-associated gene(s).

2.4. Protein interaction network analysis

To further characterize the potential involvement of TFAM and POLG in leprosy, we constructed the interaction network using a high-confidence gene and protein interaction database GeneMANIA (http://www.genemania.org; [29]). Firstly, we constructed the interaction network with only *TFAM* and *POLG* as the input, to extract direct interactors and involved pathways of these two genes in an unsupervised manner. Then, the recognized leprosy risk genes compiled in our recent study (Ref. [30] and references therein) were added, together with *TFAM* and *POLG*, as the input for network construction, to investigate the potential involvement of *TFAM* and *POLG* in network comprising all leprosy-associated genes.

2.5. eQTL and differential mRNA expression analysis

We performed eQTL analysis to investigate whether the *TFAM* and *POLG* tag SNPs affect gene expression in human blood, skin and nerve tissues using the publically available expression datasets Genotype-Tissue Expression project (GTEx, http://www.gtexportal.org/home/; [31]). Moreover, we retrieved a microarray data GSE74481 from Gene Expression Omnibus dataset (GEO) regarding leprotic skin lesions [32]. This dataset contains skin biopsies of 24 MB (10 mid-borderline leprosy [BB] + 10 borderline lepromatous [BL] + 4 LL), 20 PB (10 TT + 10 borderline-tuberculoid [BT]), 14 type I reaction (R1) patients and 10 type II reaction (R2) patients, as well as normal skin biopsies from 9 healthy individuals [32].

2.6. Statistical analysis

Deviation from the Hardy-Weinberg equilibrium (HWE), allele frequencies of all SNPs, haplotype comparisons were performed using PLINK v1.07 [33] or SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Pairwise LD was determined using Haploview 4.2 [34]. Power calculations were performed using Quanto software [35]. The *P*-values of eQTL analyses were estimated by the standard linear regression. The interactive web tool GEO2R (https://www.ncbi.nlm.nih.gov/geo/info/geo2r.html), which uses the GEOquery and limma (Linear Models for Microarray Analysis) R package, was used to compare the differential mRNA expression levels of *TFAM* and *POLG* in leprosy patients compared with controls based on the reported dataset GSE74481 [32].

The variant with an allele frequency <1% in case and control groups identified in Sample II was regarded as rare variant. Missense variants were rated as damaging when at least two of five prediction algorithms (SIFT [36,37], PolyPhen2 HumDiv, PolyPhen2 HumVar [38], LRT [39] and MutationTaster [40]) suggesting a potential deleterious effect. The gene-based burden testing, based on rare damaging variants, was performed using PLINK/seq v0.09 (https://atgu.mgh.harvard.edu/plinkseq/). A *P*-value < 0.05 was considered as statistically significant. Bonferroni corrected *P*-value was used for multiple comparisons.

3. Results

3.1. TFAM rs1049432 and POLG rs3176238 conferred genetic susceptibility to leprosy

The MAF of the 7 tag SNPs in *TFAM* and *POLG* ranged from 11.2% to 49.5% in our Sample I (Table 1). Assuming an odds ratio (OR) value as 1.6 for risk allele, the power was expected to be above 89.5% (Fig. S1). All the tag SNPs were in HWE in both controls and cases (Table S2). The observed distributions of allele and genotype frequencies in leprosy patients and controls were summarized in Tables 1 and S3, respectively. The *TFAM* rs1049432 was associated with leprosy *per se* (T allele, $P = 0.002$, OR [95%CI] = 0.671 [0.523–0.860]) and MB (T allele, $P = 0.007$, OR [95%CI] = 0.651 [0.477–0.889]). The significant association of leprosy *per se* appeared to be caused by the skewing effect of the MB patients. The frequency of *POLG* rs3176238-T allele was smaller in MB patients than in controls (T allele, $P = 0.006$, OR [95%CI] = 0.740 [0.598–0.917]). Recently, Gaschignard and colleagues performed association study using leprosy clinical forms as phenotypes (MB and PB) and they found that this is a powerful strategy to identify genetic factors affecting disease polarization [41]. However, we found no association of *TFAM* and *POLG* SNPs with disease polarization when we compared MB patients with PB patients (Tables 1, S3 and S4). We also performed association analyses using unconditional logistic regression with an adjustment for sex, to test whether gender affects the above results. The *TFAM* rs1049432, but not *POLG* rs3176238, was associated with leprosy *per se* and MB ($P < 0.007$) at the allelic level after Bonferroni correction (Table S4).

We constructed the LD map of these tag SNPs of each gene in the cases, controls, CHB and Southern Han Chinese (CHS) populations from 1000 Genomes dataset [42], and observed similar LD structures for these populations (Fig. S2). In the sliding window-based haplotype analysis, we found significant haplotypic associations of 2-SNPs (rs10826179–rs1049432 of *TFAM* and rs3176238–rs2247233 of *POLG*) and 3-SNPs (rs11006127–rs10826179–rs1049432 of *TFAM*) with leprosy (Fig. 2). The associations survived Bonferroni correction for multiple comparisons.

3.2. Leprosy-associated tag SNPs affected gene expression of TFAM and POLG in human tissues

We tested the eQTL effect of the leprosy-associated SNPs in human blood, skin and nerve tissues using GTEx datasets [31]. We

Table 1
Comparison of allele frequencies of 7 SNPs of *TFAM* and *POLG* in 527 leprosy patients and 583 healthy controls from the Yuxi Prefecture, Yunnan, China.

SNP ID	Allele	Gene	F_U	Leprosy per se versus controls				MB versus controls				PB versus controls				MB versus PB		
				F_A	P^*	OR	95% CI	F_A	P^*	OR	95% CI	F_A	P^*	OR	95% CI	P^*	OR	95% CI
rs10826175	G/A	*TFAM*	0.476	0.495	0.379	1.078	0.912–1.275	0.458	0.482	0.929	0.758–1.140	0.536	0.026	1.271	1.029–1.570	0.012	0.731	0.573–0.933
rs11006127	A/C	*TFAM*	0.323	0.325	0.916	1.010	0.845–1.207	0.357	0.154	1.168	0.944–1.445	0.288	0.167	0.850	0.676–1.070	0.017	1.373	1.058–1.782
rs10826179	G/A	*TFAM*	0.483	0.467	0.432	0.935	0.790–1.106	0.487	0.880	1.016	0.829–1.245	0.444	0.138	0.852	0.689–1.053	0.156	1.192	0.935–1.521
rs1049432	T/G	*TFAM*	0.158	0.112	**0.002**	0.671	0.523–0.860	0.109	**0.007**	0.651	0.477–0.889	0.115	0.023	0.693	0.504–0.953	0.749	0.939	0.639–1.380
rs1138465	G/A	*POLG*	0.484	0.443	0.056	0.849	0.718–1.004	0.430	0.037	0.805	0.657–0.987	0.458	0.333	0.901	0.730–1.113	0.365	0.893	0.700–1.140
rs3176238	T/C	*POLG*	0.387	0.341	0.025	0.820	0.689–0.976	0.318	**0.006**	0.740	0.598–0.917	0.366	0.434	0.917	0.737–1.140	0.101	0.808	0.625–1.043
rs2247233	A/G	*POLG*	0.448	0.419	0.168	0.888	0.750–1.051	0.399	0.054	0.817	0.665–1.004	0.442	0.803	0.973	0.788–1.203	0.162	0.839	0.657–1.073

F_U: minor allele frequency in controls; F_A: minor allele frequency in cases; MB: multibacillary leprosy; PB: paucibacillary leprosy; OR: odds ratio; 95%CI: 95% confidence interval.
* *P*-values were calculated by using the Chi-square test. *P*-values less than 0.0071 (Bonferroni correction: 0.05/7) were marked in bold.

found that rs1049432 was a significant *cis* eQTL for *TFAM* in nerve tissue ($P = 1.20 \times 10^{-12}$, Fig. 3A) and rs3176238 was a significant *cis* eQTL for *POLG* in nerve tissue ($P = 3.90 \times 10^{-13}$, Fig. 3B) and skin tissue ($P = 2.50 \times 10^{-11}$, Fig. 3C). In addition, we found a significantly differential mRNA expression of *POLG* (in MB, PB and R1 patients), but not *TFAM*, in leprotic skin lesions compared with those skin biopsies of healthy individuals from dataset GSE74481 [32] (Fig. 3D and E).

3.3. Protein interaction network analysis of TFAM, POLG and reported leprosy risk genes

We used GeneMANIA prediction server [29] to recognize potential interaction between TFAM and POLG, and found that TFAM could indirectly interact with POLG (Fig. 4). These two proteins could also physically interact with the other proteins that are actively involved in mtDNA replication, such as POLG2, TFB1M, TFB2M and POLRMT [43]. In addition, we found that TFAM and POLG could participate in the molecular network that was composed of previously reported leprosy susceptibility genes (Ref. [30] and references therein) (Fig. S3).

3.4. Deep sequencing of the TFAM and POLG exons identified no causal variants

To identify whether there are potentially causal variants in the coding regions of *TFAM* and *POLG* that could affect the risk of leprosy, we performed targeted gene sequencing to screen the entire exons and flanking region of *TFAM* and *POLG* in Sample II from the Wenshan Prefecture.

For 798 leprosy patients and 990 healthy controls, the average sequencing coverage was 160×, and the mean depth of on-target reads of *TFAM* and *POLG* were 68× and 45×, respectively. A total of 16 variants (including 2 common variants and 14 rare variants) of *TFAM* and 97 variants (including 6 common variants and 91 rare variants) of *POLG* were found in Sample II. We constructed the LD map of these SNPs with a MAF > 0.01 in the cases and controls for each gene. Consistent with the pattern in Sample I, we observed a similar LD structure in the cases and controls though the SNPs were different (Fig. S4). These results indicated that our case and control populations had no population substructure.

Variants rs1937 of *TFAM* and rs61756401 of *POLG* were significantly associated with leprosy when the ExAC dataset was used as control samples to compare with leprosy patients in Sample II (rs1937: $P = 1.143 \times 10^{-26}$, OR [95%CI] = 0.388 [0.325–0.464]; rs61756401: $P = 0.003$, OR [95%CI] = 1.761 [1.233–2.514]; Table 2). We observed same OR directions for rs1937 and rs61756401 when we compared leprosy patients with controls in Sample II though *P* values did not reach a significant level. We performed LD analysis based on the CHB dataset from 1000 Genomes dataset (each SNP with a MAF > 0.01) [42], to find out whether the leprosy-associated SNP in Sample I (rs1049432 in *TFAM* and rs3176238 in *POLG*) was linked with any SNP identified in Sample II. We found that rs1049432 had a very high LD ($r^2 = 1$) with

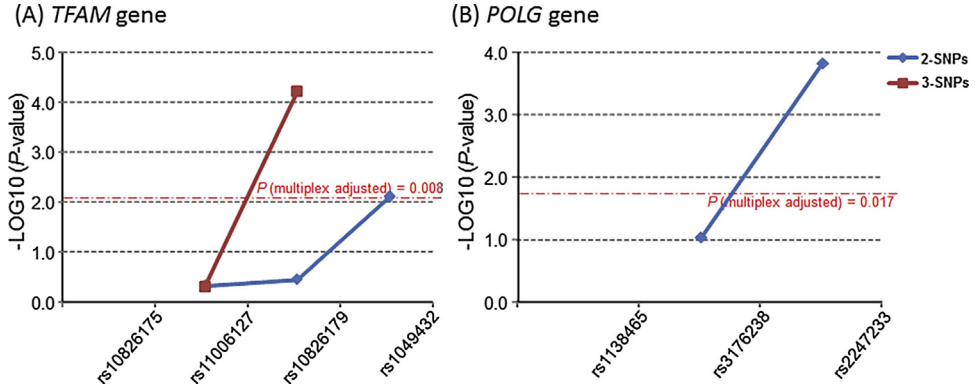

Fig. 2. Sliding window haplotype analysis of 4 SNPs in *TFAM* and 3 SNPs in *POLG* in the Yuxi sample. A total of 6 windows for *TFAM* and 3 windows for *POLG* were observed. Results of 2-SNPs, 3-SNPs windows for *TFAM* (A) and 2-SNPs windows for *POLG* (B) were shown here. A *P*-value < 0.008 (0.05/6) in *TFAM* and a *P*-value < 0.017 (0.05/3) in *POLG* were regarded as statistically significant after Bonferroni correction.

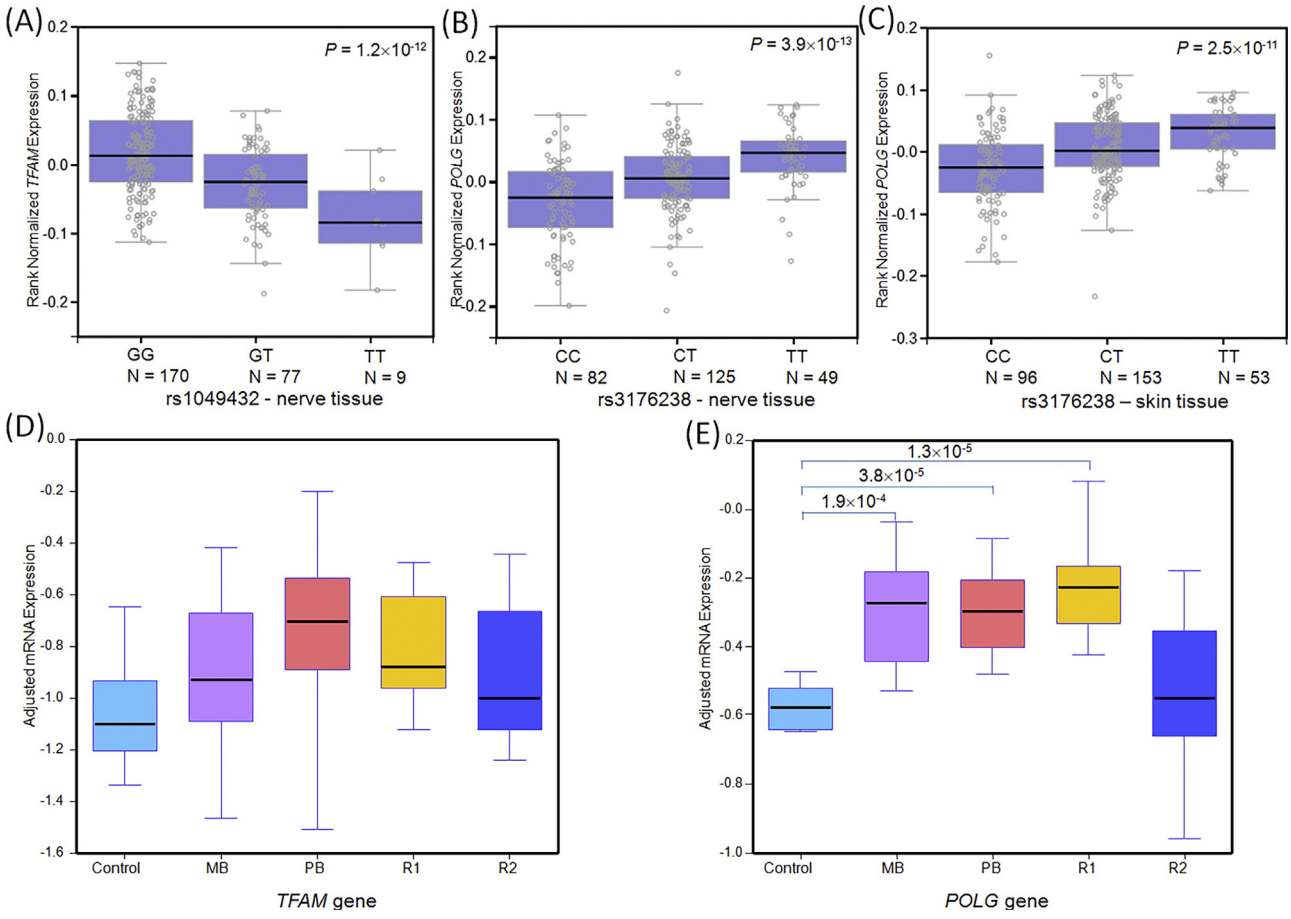

Fig. 3. Gene expression analysis. eQTL analysis of the *TFAM*-rs1049432 (A) and *POLG*-rs3176238 (B, C) in human tissues using the GTEx [31]. Differential mRNA expression levels of *TFAM* (D) and *POLG* (E) in leprotic skin lesions were performed using microarray expression data GSE74481 [32]). The dataset GSE74481 contains skin biopsies of 24 MB (10 mid-borderline leprosy [BB] + 10 borderline lepromatous [BL] + 4 LL), 20 PB (10 TT + 10 borderline-tuberculoid [BT]), 14 type I reaction (R1) patients and 10 type II reaction (R2) patients, as well as normal skin biopsies from 9 healthy individuals [32].

rs1937 (Fig. S5). Therefore, based on the linkage pattern, the leprosy-associated SNP (rs1049432) in Sample I could be validated by the linked SNP (rs1937) in Sample II. The two leprosy-associated SNPs in *POLG* (rs3176238 and rs61756401) that were identified in Sample I and Sample II, respectively, were not linked in the CHB dataset ($r^2 = 0.02$). Although rs3176238 was linked with rs2302084 ($r^2 = 0.81$), the latter had no association with leprosy (leprosy patients in Sample II versus the ExAC data, $P = 0.399$; Table 2). None of rare variants with a MAF < 0.01 in the coding region of *TFAM* and *POLG* were associated with leprosy. However, 25 variants were predicted to be damaging in all Sample II subjects. Among them, 5 variants with a putative pathogenic effect were only presented in patients (Table S5). The gene-based burden test showed that *TFAM* and *POLG* had no enrichment of damaging variants compared with controls ($P > 0.05$, Table S6). Apparently, the risk effect was most likely contributed by a remote regulatory locus that was signposted by the leprosy-associated tag SNPs, similar to the finding of long-range gene regulation of *IRX3* in type 2 diabetes [44] and other good cases for long-range regulatory elements [45,46].

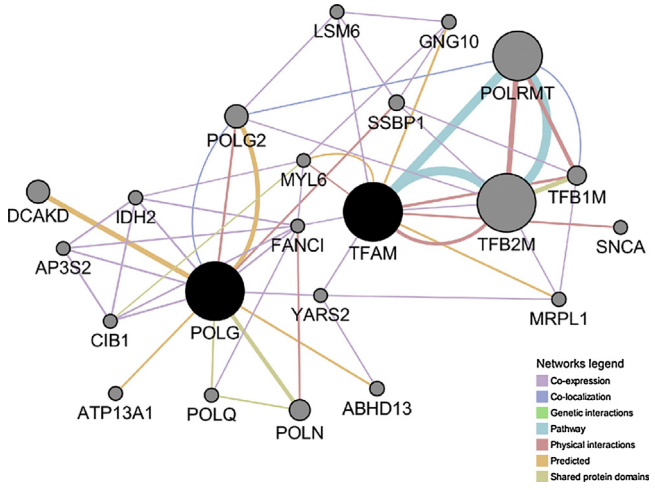

Fig. 4. Protein interaction network of TFAM and POLG using the GeneMANIA prediction server [29]. We entered the TFAM and POLG to probe the potential interactions, including co-expression, genetic and physical interaction networks. The lines with different colors represent different correlations, in which the thickness corresponds to the degree of correlation.

4. Discussion

Increasing evidence suggests that mitochondrion-related genes play important roles of in antimicrobial immune response and energy production [6,47]. Under an evolutionary hypothesis of host energy dependence of *M. leprae* due to its eroded genome, we and others speculated that genetic variants of host mitochondrion-related genes would affect risk to leprosy. Indeed, there are many reports for association of mitochondrion-related genes with leprosy [10–12,48,49], albeit there is occasionally negative report [13]. The first leprosy genome-wide association study (GWAS)

Table 2
SNPs with a MAF > 0.01 in the exon and flank regions of *TFAM* and *POLG* in 798 leprosy cases and 990 controls from the Wenshan Prefecture, Yunnan, China.

Chr	Position	SNP ID	Ref/ Alt	Gene	Residue change	Function	Damaging[a]	Allele counts in patients	Allele counts in controls	P^*	OR (95% CI)	Allele counts in ExAC-EAS	P^*	OR (95%CI)
chr15	89860786	rs2307438	A/C	*POLG*	–	intron	–	577/1596	710/1980	0.861	1.013 (0.883–1.162)	3046/8594	0.589	1.031 (0.923–1.153)
chr15	89862341	rs2302084	A/G	*POLG*	–	intron	–	534/1596	658/1978	0.915	1.009 (0.877–1.160)	2793/8628	0.399	1.050 (0.938–1.177)
chr15	89864088	rs201477273	G/A	*POLG*	p.R964C	missense	Damaging	22/1596	22/1980	0.542	1.244 (0.686–2.255)	80/8646	0.099	1.497 (0.931–2.406)
chr15	89864294	rs2074883	T/C	*POLG*	–	intron	–	65/1596	63/1976	0.174	1.289 (0.906–1.835)	322/8636	0.520	1.096 (0.838–1.444)
chr15	89869833	rs55962804	C/T	*POLG*	–	intron	–	33/1596	34/1968	0.460	1.201 (0.740–1.948)	145/8628	0.297	1.235 (0.843–1.810)
chr15	89872249	rs61756401	C/T	*POLG*	p.K316	synonymous	–	41/1596	34/1980	0.079	1.509 (0.953–2.389)	127/8608	0.003	1.761 (1.233–2.514)
chr10	60145342	rs1937	G/C	*TFAM*	p.S12T	missense	Tolerated	215/1596	302/1980	0.138	0.865 (0.716–1.045)	481/1680	1.143×10^{-26}	0.388 (0.325–0.464)
chr10	60147934	rs2277256	T/C	*TFAM*	–	intron	–	89/1596	101/1980	0.549	1.099 (0.820–1.473)	456/8336	0.857	1.021 (0.808–1.289)

Chr, Chromosome; Ref, Reference allele; Alt, Alternate allele; ExAC-EAS, 4327 East Asian (EAS) individuals in the ExAC database [28]; OR, Odds ratio; 95%CI, 95% confidence intervals; –, no data available.

[a] Missense variants are rated as damaging when at least two of five prediction algorithms (SIFT [36,37], PolyPhen2 HumDiv, PolyPhen2 HumVar [38], LRT [39] and MutationTaster [40]) suggesting a potential deleterious effect, otherwise the variants are rated as tolerated.

[*] *P*-values were calculated by using the Fisher's exact test.

highlighted the mitochondrion-related gene *LRRK2* that was associated with leprosy [50], though subsequent analyses showed that the *LRRK2* risk SNPs or their effects were inconsistent in different populations [11,48,51–53]. Recently, we provided direct genetic evidence that mitochondrion-related genes *OPA1* [10], *LRRK2* [11], *PINK1* and *PARL* [12] were associated with leprosy in Han Chinese populations from Southwest China. The mitochondrion-related gene *SOD2* was reported to be associated with leprosy in Brazilian [49]. In addition, Guerreiro and colleagues reported that mitochondrial metabolism genes, which were mainly involved in oxidative phosphorylation pathway, were down-regulated in *M. leprae* infected Swann cells [54]. These findings supported our speculation that mitochondrion-related genes had an active role in host response to *M. leprae* and affected the risk of infection. In this study, we presented further genetic evidence that mtDNA replication-related genes *TFAM* and *POLG* were associated with leprosy susceptibility.

Among the 7 tag SNPs studied in Sample I, *TFAM* rs1049432 and *POLG* rs3176238 were associated with MB after multiple corrections. We further performed eQTL analysis to elucidate whether these two risk variants altered the *TFAM* and *POLG* mRNA expression. We found that rs1049432 was a *cis* eQTL for *TFAM* mRNA expression in human nerve tissue, and rs3176238 was a *cis* eQTL for *POLG* mRNA expression in both human nerve and skin tissues. Concordantly, we found a significantly differential mRNA expression of *POLG*, but not *TFAM*, between leprotic skin lesions of patients (leprosy subtypes) and control tissues based on the re-analysis of the reported dataset GSE74481 [32] (Fig. 3). However, when we used the RegulomeDB database (http://www.regulomedb.org/index; [55]) to further annotate the above two leprosy-associated SNPs, none of them had potential functions such as transcription factor binding site and DNase hypersensitivity (Table S7). To pinpoint potential causal variants in coding regions of *TFAM* and *POLG* with leprosy, we performed a targeted sequencing for both genes, and found that two coding region variants (rs1937 of *TFAM* and rs61756401 of *POLG*) showing an association with leprosy when we compared the leprosy sample with the ExAC-EAS data (Table 2). Based on the LD structure in the CHB dataset [42], rs1937 was highly linked with rs1049432, which suggested that the association of rs1049432 with leprosy as identified in Sample I could be replicated in Sample II albeit rs1049432 was not genotyped in Sample II subjects.

Previous studies showed that TFAM, TFB1M and TFB2M are key transcription specificity factors required by the mitochondrial RNA polymerase (POLRMT) in the initiation of mtDNA transcription [56]. Evidence from *in vivo* study showed that mtDNA copy number regulation was directly dependent on the TFAM protein levels in mouse tissues and embryos [19]. Moreover, DNA methylation and demethylation of *POLG* could regulate mtDNA copy number in mouse [57] and human stem and cancer cells [58], although there are some controversies [59]. We performed the protein interaction network, which showed that the TFAM and POLG interacted with the other mtDNA replication-related proteins (Fig. 4). We previously reported a differential mtDNA copy number in the leprosy patients stratified by clinical subtypes [8], but it would be difficult to confirm the direct links among the mtDNA copy number, leprosy subtypes and/or risk variants of *TFAM* and *POLG*. Further studies with an enlarged sample size and more tissues including leprotic skin lesions and nerve tissues should be carried out to clarify this issue.

Leprosy is also regarded as a neurological disease because of nerve damage in patients [2]. Many leprosy risk genes (such as *PARK2* [60], *PINK1* [12] and *CHF* [61]) were found to be associated with neurodegenerative diseases including PD [62,63] and AD [64]. PINK1 and Parkin (encoded by the *PARK2* gene) could suppress the presentation of mitochondrial antigens on the MHC Class I molecules [65]. Parkin also played important roles in ubiquitin-mediated autophagy during *M. tuberculosis* infection, and it was

associated with mitochondrial homeostasis during host-pathogen interaction [66]. We noticed that *TFAM* and *POLG* genetic variants have been reported to be associated with AD and/or PD [18,22,23]. It would be rewarding to characterize the potential roles of *TFAM* and *POLG* variants in the nervous system during *M. leprae* infection.

In summary, two tag SNPs of the mtDNA transcription and replication genes *TFAM* and *POLG* were associated with leprosy, and the risk alleles affected the expression of these two genes. Variants in the coding regions of *TFAM* and *POLG* gene might not play an active role in leprosy. It should be mentioned that the lack of overlapped genotyped loci in the two independent samples is a limitation of this study. Future studies are needed to validate the association in independent populations and to explore the underlying mechanism.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2017.09.001.

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Online supplementary data

Table S1. Primers used for genotyping

SNPs	Primer (5'-3')
	Forward: ATATATGCCTCCGTCAACCTTAT
	Reverse: AAAGTAACTTTTGAACGTTAACTCTTAGA
rs10826175	Probe: $(gact)_{11}$GAAAATTAAAAATTATAAATGAACT
	Forward: AAAAGAAAAATTTGTCAGTAGCATG
	Reverse: TATGTATTTCCTTCTCTCTAGTCCCA
rs11006127	Probe: act$(gact)_{13}$CCCATTGCTGTTGCTTTTGTTCAGG
	Forward: TGCAATATTTTCTATGGGGAAT
	Reverse: ATTGACAAGCTTTCTTTTTCTTTG
rs10826179	Probe: act$(gact)_{9}$TATCTTTTAATAACATTTTGGTTAT
	Forward: AAGCAAATGTGAATCATTTTACCT
	Reverse: CCTCCATAATATAAGGAAACAAGAGTAC
rs1049432	Probe: t$(gact)_{12}$TGACAAAGGTAAATCAGACTATGAA
	Forward: TGGGCTCCAGACAGAAGC
	Reverse: AATCTTGTCATAGGTGAACCTGAT
rs1138465	Probe: t$(gact)_{6}$CACTGACCTCTTCATTTTCATCTTC
	Forward: TAATTTCAGTTTATAAGGTGCCCT
	Reverse: GACAGGTACAGGTAGAATATTTGAAG
rs3176238	Probe: act$(gact)_{9}$ATTTGAAGCTCTGCTGCCTTCATTT
	Forward: TAATAGCGTCATGAGAAGTTAGTCTG
	Reverse: TTTATGGGAAAGGCTGGG
rs2247233	Probe: ct$(gact)_{8}$TGGGGAGCTATTAATTATTTTGGGC

Note: (gact)n, n repeats of “gact”

Table S2. Hardy-Weinberg equilibrium test of the studied SNPs in 527 leprosy patients and 583 controls from Yuxi, Yunnan Province, China

SNP	Gene	*P*-value (patients)	*P*-value (controls)
rs10826175	*TFAM*	0.10	0.09
rs11006127	*TFAM*	0.27	0.15
rs10826179	*TFAM*	0.38	0.80
rs1049432	*TFAM*	0.27	0.43
rs1138465	*POLG*	0.60	0.80
rs3176238	*POLG*	0.77	0.73
rs2247233	*POLG*	0.59	0.45

Table S3. Comparison of the genotype frequencies of 7 SNPs in 527 leprosy patients and 583 healthy controls from Yuxi, Yunnan Province, China

SNP ID	Allele	TEST	No. of Controls	Leprosy versus controls		MB versus controls		PB versus controls		MB versus PB
				No.	*P**	No.	*P**	No.	*P**	*P**
rs10826175	G/A	GENO	140/265/167	138/242/143	0.696	63/126/86	0.791	75/116/57	0.098	0.051
	G/A	DOM	405/167	380/143	0.497	189/86	0.536	191/57	0.067	0.034
	G/A	REC	140/432	138/385	0.468	63/212	0.617	75/173	0.085	0.057
rs11006127	A/C	GENO	68/237/273	61/219/245	0.972	42/114/121	0.334	19/105/124	0.210	0.024
	A/C	DOM	305/273	280/245	0.851	156/121	0.330	124/124	0.466	0.148
	A/C	REC	68/510	61/464	0.940	42/235	0.165	19/229	0.078	0.007
rs10826179	G/A	GENO	132/289/151	119/251/154	0.528	67/135/74	0.895	52/116/80	0.230	0.355
	G/A	DOM	421/151	370/154	0.270	202/74	0.898	168/80	0.087	0.172
	G/A	REC	132/440	119/405	0.885	67/209	0.700	52/196	0.506	0.367
rs1049432	T/G	GENO	17/149/414	9/99/416	0.008	3/54/219	0.027	6/45/197	0.052	0.472
	T/G	DOM	166/414	108/416	**0.002**	57/219	0.016	51/197	0.016	1.000
	T/G	REC	17/563	9/515	0.184	3/273	0.144	6/242	0.682	0.319
rs1138465	G/A	GENO	134/293/153	100/266/160	0.152	46/147/85	0.073	54/119/75	0.522	0.285
	G/A	DOM	427/153	366/160	0.136	193/85	0.199	173/75	0.254	0.934
	G/A	REC	134/446	100/426	0.096	46/232	0.027	54/194	0.676	0.127
rs3176238	T/C	GENO	85/281/217	59/240/226	0.079	25/127/126	0.019	34/113/100	0.676	0.186
	T/C	DOM	366/217	299/226	0.048	152/126	0.023	147/100	0.376	0.264
	T/C	REC	85/498	59/466	0.099	25/253	0.022	34/213	0.760	0.084
rs2247233	A/G	GENO	121/277/181	89/262/174	0.247	41/139/97	0.095	48/123/77	0.854	0.328
	A/G	DOM	398/181	351/174	0.504	180/97	0.272	171/77	0.952	0.335
	A/G	REC	121/458	89/436	0.095	41/236	0.033	48/200	0.614	0.165

MB – multibacillary leprosy; PB – paucibacillary leprosy; GENO: genotypic; DOM: dominant model; REC: recessive model

* *P*-values less than 0.05/7=0.0071 (Bonferroni correction) were marked in bold.

Table S4. Genotype and allele frequencies of 7 SNPs in 527 leprosy patients and 583 healthy controls (adjusted by sex)

SNP	Genotype /Allele	Patients versus controls		MB versus controls		PB versus controls		MB versus PB	
		P-value*	OR (95% CI)	*P*-value*	OR (95% CI)	*P*-value*	OR (95% CI)	*P*-value*	OR (95% CI)
rs10826175	GG	reference		reference		reference		reference	
	GA	0.522	0.899 (0.648-1.247)	0.433	1.172 (0.788-1.745)	0.057	0.669 (0.442-1.013)	0.017	0.560 (0.348-0.900)
	AA	0.683	0.941 (0.700-1.263)	0.781	1.053 (0.730-1.521)	0.322	0.835 (0.584-1.194)	0.237	0.776 (0.510-1.181)
	G allele	reference		reference		reference		reference	
	A allele	0.536	0.948 (0.800-1.123)	0.364	1.100 (0.896-1.351)	0.051	1.112 (1.000-1.237)	0.014	0.737 (0.577-0.940)
rs11006127	AA	reference		reference		reference		reference	
	AC	0.878	1.031 (0.695-1.530)	0.306	0.792 (0.506-1.238)	0.123	1.556 (0.888-2.728)	0.020	2.046 (1.119-3.741)
	CC	0.953	1.102 (0.686-1.493)	0.160	0.728 (0.468-1.133)	0.096	1.602 (0.920-2.788)	0.007	2.287 (1.258-4.160)
	A allele	reference		reference		reference		Reference	
	C allele	0.968	0.996 (0.832-1.193)	0.154	0.856 (0.691-1.060)	0.177	1.173 (0.930-1.478)	0.015	1.383 (1.065-1.796)
rs10826179	GG	reference		reference		reference		reference	
	GA	0.397	1.157 (0.826-1.620)	0.918	0.979 (0.652-1.470)	0.161	1.354 (0.887-2.067)	0.163	1.411 (0.870-2.287)
	AA	0.893	0.979 (0.724-1.325)	0.744	0.942 (0.657-1.350)	0.927	1.018 (0.690-1.503)	0.620	1.118 (0.720-1.736)
	G allele	reference		reference		reference		reference	
	A allele	0.365	0.962 (0.884-1.047)	0.988	0.998 (0.814-1.225)	0.129	1.179 (0.953-1.460)	0.152	1.195 (0.936-1.525)
rs1049432	TT	reference		reference		reference		reference	
	TG	0.100	1.997 (0.876-4.552)	0.075	3.085 (0.891-10.681)	0.480	1.409 (0.544-3.646)	0.275	0.458 (0.113-1.863)
	GG	0.508	1.333 (0.569-3.124)	0.249	2.111 (0.593-7.516)	0.840	0.903 (0.334-2.439)	0.247	0.425 (0.100-1.806)
	T allele	reference		reference		reference		reference	
	G allele	**0.002**	1.497 (1.166-1.923)	**0.007**	1.538 (1.125-2.103)	0.024	1.447 (1.051-1.992)	0.762	0.942 (0.641-1.384)
rs1138465	GG	reference		reference		reference		reference	
	GA	0.063	1.385 (0.983-1.952)	0.035	1.587 (1.033-2.438)	0.403	1.197 (0.785-1.827)	0.266	0.752 (0.456-1.242)

	AA	0.237	1.206 (0.884-1.643)	0.063	1.449 (0.980-2.142)	0.944	1.014 (0.691-1.488)	0.112	0.687 (0.433-1.091)
	G allele	reference		reference		reference		reference	
	A allele	0.067	1.171 (0.989-1.386)	0.055	1.222 (0.996-1.500)	0.384	1.099 (0.889-1.359)	0.351	0.890 (0.698-1.136)
rs3176238	TT	reference		reference		reference		reference	
	TC	0.048	1.473 (1.004-2.162)	0.010	1.931 (1.172-3.181)	0.635	1.119 (0.702-1.785)	0.069	0.584 (0.327-1.043)
	CC	0.334	1.204 (0.826-1.756)	0.103	1.510 (0.921-2.477)	0.952	0.986 (0.624-1.557)	0.146	0.653 (0.367-1.160)
	T allele	reference		reference		reference		reference	
	C allele	0.032	1.211 (1.017-1.443)	0.009	1.331 (1.073-1.650)	0.519	1.075 (0.863-1.338)	0.095	0.804 (0.623-1.039)
rs2247233	AA	reference		reference		reference		reference	
	AG	0.156	1.264 (0.914-1.748)	0.075	1.452 (0.963-2.189)	0.593	1.115 (0.748-1.661)	0.251	0.754 (0.465-1.221)
	GG	0.150	1.289 (0.912-1.823)	0.048	1.548 (1.003-2.388)	0.795	1.059 (0.688-1.628)	0.138	0.678 (0.406-1.133)
	A allele	reference		reference		reference		reference	
	G allele	0.194	1.119 (0.944-1.326)	0.079	1.204 (0.979-1.481)	0.864	1.019 (0.823-1.261)	0.153	0.836 (0.654-1.069)

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. *P*-values less than 0.0071 (Bonferroni correction: 0.05/7) were marked in bold.

Table S5. The list of SNPs in the exon and flank regions in the *TFAM* and *POLG* genes in 798 leprosy cases and 990 controls from Wenshan, Yunnan by using the next-generation sequencing technologies

Chr	Position	rsID	Ref	Alt	Gene	Residue change	Function	Damaging predication #	Allele counts in patients	Allele counts in controls	*P* *	OR	Allele counts in ExAC-EAS	*P* *	OR
chr15	89859992	rs200788482	G	A	*POLG*	p.1237P>L	missense	Tolerated	0/1596	1/1980	1.000	0.413	NA	-	-
chr15	89860046	.	T	C	*POLG*	p.1219D>G	missense	Damaging	0/1596	2/1980	0.506	0.248	0/8642	-	-
chr15	89860052	rs199751339	G	A	*POLG*	p.1217A>V	missense	Damaging	3/1596	1/1980	0.330	3.727	5/8642	0.115	3.253
chr15	89860076	.	T	C	*POLG*	-	intron	-	1/1596	0/1980	0.446	3.724	3/8584	0.495	1.793
chr15	89860574	.	A	G	*POLG*	-	intron	-	0/1596	2/1980	0.506	0.248	NA	-	-
chr15	**89860640**	**.**	**T**	**C**	***POLG***	**p.1204T>A**	**missense**	**Damaging**	**1/1596**	**0/1980**	**0.446**	**3.724**	**NA**	**-**	**-**
chr15	89860652	.	G	T	*POLG*	p.1200P>T	missense	Damaging	0/1596	1/1980	1.000	0.413	NA	-	-
chr15	89860690	rs199678775	C	T	*POLG*	p.1187R>Q	missense	Tolerated	0/1596	1/1980	1.000	0.413	0/8654	-	-
chr15	89860761	.	C	T	*POLG*	p.1163M>I	missense	Damaging	0/1596	1/1980	1.000	0.413	NA	-	-
chr15	89861757	.	A	G	*POLG*	-	intron	-	0/1596	1/1980	1.000	0.413	NA	-	-
chr15	89861765	rs200309191	C	T	*POLG*	-	intron	-	3/1596	2/1980	0.662	1.863	0/8618	-	-
chr15	**89861794**	**.**	**G**	**T**	***POLG***	**p.1154Q>K**	**missense**	**Damaging**	**1/1596**	**0/1980**	**0.446**	**3.724**	**NA**	**-**	**-**
chr15	89861810	rs374937961	G	A	*POLG*	p.1148R>R	synonymous	-	1/1596	0/1980	0.446	3.724	0/8638	-	-
chr15	89861830	rs2307442	G	A	*POLG*	p.1142R>W	missense	Damaging	0/1596	3/1980	0.258	0.177	0/8646	-	-
chr15	89861840	.	G	A	*POLG*	p.1138R>R	synonymous	-	6/1596	2/1980	0.150	3.732	NA	-	-
chr15	**89861857**	**.**	**T**	**C**	***POLG***	**p.1133I>V**	**missense**	**Damaging**	**1/1596**	**0/1980**	**0.446**	**3.724**	**NA**	**-**	**-**
chr15	**89861919**	**.**	**A**	**C**	***POLG***	**p.1112M>R**	**missense**	**Damaging**	**2/1596**	**0/1980**	**0.199**	**6.210**	**NA**	**-**	**-**
chr15	89862159	.	T	A	*POLG*	-	spliceSite	-	2/1596	0/1980	0.199	6.210	NA	-	-
chr15	89862161	.	C	G	*POLG*	-	spliceSite	-	2/1596	0/1978	0.199	6.204	NA	-	-
chr15	89862255	rs200759978	C	T	*POLG*	p.1060K>K	synonymous	-	0/1596	1/1980	1.000	0.413	NA	-	-
chr15	89862259	rs201192905	T	C	*POLG*	p.1059N>S	missense	Damaging	0/1596	2/1980	0.506	0.248	2/8652	-	-
chr15	89862354	.	A	T	*POLG*	-	intron	-	2/1596	0/1980	0.199	6.210	NA	-	-

chr15	89862450	.	G	A	*POLG*	-	intron	-	1/1596	0/1980	0.446	3.724	1/8652	0.287	5.424
chr15	89862612	rs2307432	G	A	*POLG*	-	intron	-	0/1596	1/1980	1.000	0.413	1/8614	-	-
chr15	89862631	.	C	T	*POLG*	-	intron	-	0/1596	1/1978	1.000	0.413	9/8606	-	-
chr15	89862638	.	G	T	*POLG*	-	intron	-	0/1596	2/1978	0.506	0.248	NA	-	-
chr15	89863964	.	G	A	*POLG*	-	intron	-	0/1596	1/1976	1.000	0.412	NA	-	-
chr15	89863984	.	G	A	*POLG*	-	intron	-	1/1596	0/1978	0.447	3.720	0/8622	-	-
chr15	89864074	.	C	T	*POLG*	p.968Q>Q	synonymous	-	0/1596	1/1980	1.000	0.413	NA	-	-
chr15	89864094	.	C	T	*POLG*	p.962A>T	missense	Damaging	7/1596	6/1980	0.582	1.449	3/8648	1.7×10^{-4}	12.695
chr15	89864143	.	A	C	*POLG*	p.945H>Q	missense	Damaging	0/1596	1/1980	1.000	0.413	2/8644	-	-
chr15	89864148	.	C	T	*POLG*	p.944E>K	missense	Damaging	0/1596	2/1980	0.506	0.248	20/8644	-	-
chr15	89864152	.	G	A	*POLG*	p.942S>S	synonymous	-	0/1596	1/1980	1.000	0.413	NA	-	-
chr15	89864366	rs377390914	G	A	*POLG*	p.908A>A	synonymous	-	0/1596	3/1976	0.258	0.177	0/8646	-	-
chr15	89864414	.	G	A	*POLG*	p.892D>D	synonymous	-	0/1596	2/1980	0.506	0.248	NA	-	-
chr15	89864489	rs201749977	A	G	*POLG*	p.867P>P	synonymous	-	1/1596	3/1978	0.633	0.413	9/8494	1.000	0.591
chr15	89864531	rs55792683	C	A	*POLG*	-	intron	-	4/1596	15/1978	0.040	0.329	53/8136	0.070	0.383
chr15	89865024	rs143810171	G	A	*POLG*	p.847A>A	synonymous	-	5/1594	8/1974	0.783	0.773	38/8654	0.672	0.713
chr15	**89865066**	**.**	**C**	**G**	***POLG***	**p.833E>D**	**missense**	**Damaging**	**1/1596**	**0/1978**	**0.447**	**3.720**	**NA**	**-**	**-**
chr15	89865921	.	G	A	*POLG*	-	intron	-	0/1596	1/1974	1.000	0.412	NA	-	-
chr15	89865973	.	C	G	*POLG*	p.809S>T	missense	Damaging	0/1596	1/1976	1.000	0.412	NA	-	-
chr15	89866734	.	A	G	*POLG*	p.722R>R	synonymous	-	1/1596	1/1980	1.000	1.241	0/8622	-	-
chr15	89867031	.	C	T	*POLG*	-	intron	-	0/1596	1/1980	1.000	0.413	2/8654	-	-
chr15	89867035	rs56411159	G	A	*POLG*	-	intron	-	5/1596	7/1980	1.000	0.886	74/8654	0.019	0.364
chr15	89867066	.	G	A	*POLG*	p.713P>S	missense	Tolerated	0/1596	1/1980	1.000	0.413	NA	-	-
chr15	89867282	rs112824476	G	A	*POLG*	-	intron	-	1/1596	0/1980	0.446	3.724	NA	-	-
chr15	89867304	.	C	G	*POLG*	-	intron	-	1/1596	3/1980	0.633	0.413	NA	-	-
chr15	89867380	rs373550219	C	T	*POLG*	p.676A>A	synonymous	-	0/1596	1/1980	1.000	0.413	0/8636	-	-
chr15	89867428	.	A	G	*POLG*	p.660C>C	synonymous	-	0/1596	2/1980	0.506	0.248	0/8592	-	-

chr15	89867443	rs543910258	C	T	*POLG*	p.655L>L	synonymous	-	2/1596	0/1980	0.199	6.210	6/8570	0.365	1.791
chr15	89868631	.	G	C	*POLG*	-	intron	-	0/1596	2/1972	0.505	0.247	NA	-	-
chr15	89868726	.	G	A	*POLG*	p.635P>L	missense	Tolerated	0/1596	2/1980	0.506	0.248	0/8584	-	-
chr15	89868734	.	G	C	*POLG*	p.632A>A	synonymous	-	1/1596	3/1980	0.633	0.413	2/8602	0.400	2.696
chr15	89868763	rs548491099	A	G	*POLG*	p.623L>L	synonymous	-	2/1596	2/1980	1.000	1.241	7/8634	0.638	1.546
chr15	89868840	.	C	T	*POLG*	p.597R>Q	missense	Damaging	0/1596	1/1978	1.000	0.413	NA	-	-
chr15	89868872	rs75149168	G	A	*POLG*	p.586T>T	synonymous	-	1/1596	1/1978	1.000	1.240	NA	-	-
chr15	89868940	.	C	T	*POLG*	-	intron	-	1/1596	4/1976	0.389	0.309	NA	-	-
chr15	89869908	.	C	T	*POLG*	p.549L>L	synonymous	-	0/1596	2/1980	0.506	0.248	1/8646	-	-
chr15	89869926	rs56349446	G	A	*POLG*	p.543V>V	synonymous	-	0/1596	1/1980	1.000	0.413	4/8648	-	-
chr15	89869955	rs201097813	T	C	*POLG*	p.534S>G	missense	Damaging	4/1596	1/1980	0.179	4.972	1/8644	0.003	21.716
chr15	89869957	.	C	T	*POLG*	p.533C>Y	missense	Tolerated	3/1596	0/1980	0.089	8.700	NA	-	-
chr15	89869974	.	G	C	*POLG*	-	intron	-	1/1596	0/1980	0.446	3.724	NA	-	-
chr15	89870008	rs56317445	G	C	*POLG*	-	intron	-	0/1596	1/1980	1.000	0.413	1/8604	-	-
chr15	89870180	rs555939259	C	T	*POLG*	p.516E>E	synonymous	-	1/1596	0/1976	0.447	3.716	2/8654	0.398	2.712
chr15	89870314	.	G	C	*POLG*	-	intron	-	2/1596	0/1976	0.200	6.198	2/8654	0.117	5.428
chr15	89870334	rs187354218	C	T	*POLG*	-	intron	-	2/1596	12/1976	0.028	0.205	87/8652	8.8×10^{-5}	0.124
chr15	89870532	.	G	A	*POLG*	p.433S>S	synonymous	-	0/1596	1/1980	1.000	0.413	NA	-	-
chr15	89871684	.	C	T	*POLG*	-	spliceSite	-	2/1596	1/1978	0.590	2.481	NA	-	-
chr15	89871739	rs529639381	C	T	*POLG*	p.400V>M	missense	Damaging	2/1594	1/1980	0.589	2.486	0/8592	-	-
chr15	89871784	.	G	C	*POLG*	-	intron	-	3/1596	1/1978	0.330	3.723	NA	-	-
chr15	89871841	.	G	A	*POLG*	-	intron	-	0/1596	1/1978	1.000	0.413	NA	-	-
chr15	89871913	.	T	C	*POLG*	-	spliceSite	-	1/1596	0/1980	0.446	3.724	NA	-	-
chr15	89872020	rs371431444	G	A	*POLG*	p.356L>L	synonymous	-	1/1596	2/1980	1.000	0.620	15/8646	0.493	0.361
chr15	89872175	.	G	A	*POLG*	p.341A>V	missense	Tolerated	1/1596	1/1980	1.000	1.241	0/8638	-	-
chr15	89872245	.	C	T	*POLG*	p.318G>S	missense	Damaging	0/1596	1/1980	1.000	0.413	1/8618	-	-
chr15	89872273	.	C	A	*POLG*	p.308Q>H	missense	Damaging	0/1596	2/1980	0.506	0.248	1/8596	-	-

chr15	89872296	.	T	A	*POLG*	p.301I>F	missense	Damaging	0/1596	1/1974	1.000	0.412	NA	-	-
chr15	89873290	.	C	A	*POLG*	-	intron	-	1/1596	0/1976	0.447	3.716	2/8634	0.399	2.706
chr15	89873418	.	G	A	*POLG*	p.250P>L	missense	Damaging	0/1596	1/1978	1.000	0.413	NA	-	-
chr15	89873437	.	G	A	*POLG*	p.244L>F	missense	Damaging	0/1596	1/1978	1.000	0.413	NA	-	-
chr15	89876284	.	G	A	*POLG*	-	intron	-	2/1592	3/1970	1.000	0.825	NA	-	-
chr15	89876296	rs553397365	T	A	*POLG*	-	intron	-	2/1596	1/1974	0.590	2.476	25/8228	0.298	0.412
chr15	89876451	.	C	T	*POLG*	p.179G>S	missense	Damaging	1/1596	1/1972	1.000	1.236	NA	-	-
chr15	89876653	rs56221189	C	A	*POLG*	p.111G>G	synonymous	-	1/1596	0/1972	0.447	3.709	NA	-	-
chr15	89876677	.	G	A	*POLG*	p.103S>S	synonymous	-	3/1596	0/1974	0.089	8.674	0/8192	-	-
chr15	89876689	.	C	T	*POLG*	p.99A>A	synonymous	-	1/1596	2/1978	1.000	0.619	NA	-	-
chr15	89876852	.	T	A	*POLG*	p.45Q>L	missense	Tolerated	0/1596	1/1980	1.000	0.413	NA	-	-
chr15	89876858	rs28567406	T	C	*POLG*	p.43Q>R	missense	Tolerated	7/1596	11/1980	0.813	0.789	NA	-	-
chr15	89876900	.	G	C	*POLG*	p.29S>C	missense	Tolerated	1/1596	2/1980	1.000	0.620	NA	-	-
chr15	89876963	.	T	C	*POLG*	p.8K>R	missense	Tolerated	0/1596	1/1980	1.000	0.413	NA	-	-
chr15	89877004	.	C	G	*POLG*	-	utr-5	-	0/1596	1/1980	1.000	0.413	0/134	-	-
chr10	60145327	rs200473819	T	C	*TFAM*	p.7M>T	missense	Tolerated	0/1596	1/1980	1.000	0.413	0/1114	-	-
chr10	60145355	.	G	T	*TFAM*	p.16R>S	missense	Tolerated	1/1596	0/1980	0.446	3.724	NA	-	-
chr10	60145369	.	T	C	*TFAM*	p.21L>P	missense	Damaging	0/1596	1/1980	1.000	0.413	NA	-	-
chr10	60145389	.	C	A	*TFAM*	p.28R>R	synonymous	-	0/1596	1/1980	1.000	0.413	0/1024	-	-
chr10	60145403	.	C	G	*TFAM*	p.32P>P	synonymous	-	0/1596	1/1978	1.000	0.413	NA	-	-
chr10	60146061	rs78325627	T	C	*TFAM*	p.61S>S	synonymous	-	1/1596	0/1980	0.446	3.724	4/8644	0.571	1.354
chr10	60146088	.	T	A	*TFAM*	p.70A>A	synonymous	-	0/1596	1/1980	1.000	0.413	NA	-	-
chr10	60148012	.	A	G	*TFAM*	p.95K>E	missense	Tolerated	1/1596	0/1980	0.446	3.724	NA	-	-
chr10	60154250	rs191580080	T	G	*TFAM*	-	intron	-	2/1596	2/1980	1.000	1.241	6/8304	0.623	1.735
chr10	60154269	.	T	C	*TFAM*	-	intron	-	0/1596	1/1980	1.000	0.413	NA	-	-
chr10	60154286	.	G	C	*TFAM*	-	intron	-	0/1596	3/1978	0.258	0.177	NA	-	-
chr10	60154711	.	G	A	*TFAM*	p.206E>E	synonymous	-	2/1596	0/1980	0.199	6.210	NA	-	-

chr10	60154714	.	C	T	*TFAM*	p.207D>D	synonymous	-	0/1596	1/1980	1.000	0.413	0/8364	-	-
chr10	60154776	.	A	C	*TFAM*	p.228K>T	missense	Tolerated	0/1596	1/1980	1.000	0.413	NA	-	-

Note: Variant with a MAF <1% in either patient or control group was regarded as rare variant. Chr, Chromosome; Ref, Reference allele; Alt, Alternate allele; ExAC-EAS, 4327 East Asian (EAS) individuals in the ExAC database [1]; OR, Odds ratio; NA, no data available.

[#] 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 scored as "tolerated".

[*] *P*-values were calculated by using the Fisher's exact test. All *P*-values were not statistically significant after Bonferroni correction between leprosy cases and healthy controls. Damaging variants that were only observed in leprosy patients were marked in bold.

Table S6. Gene-based burden test of *TFAM* and *POLG* showed no significant enrichment of damaging variants

Symbol	Locus	NVAR	*P*	DESC
TFAM	ENST00000487519	1	1.000	0/1
POLG	ENST00000442287	28	0.533	49/55

NVAR: number of variants; *P*: *P*-value based on permutation, the empirical significance; DESC: the number of case/control minor alleles. The gene-based burden test was analyzed based on the damaging variants within the target gene

Table S7. SNP annotation with known and predicted regulatory elements

SNP	Annotation
rs10826175	other
rs11006127	other
rs10826179	-
rs1049432	other
rs1138465	TF binding + any motif + DNase Footprint + DNase peak
rs3176238	other
rs2247233	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.

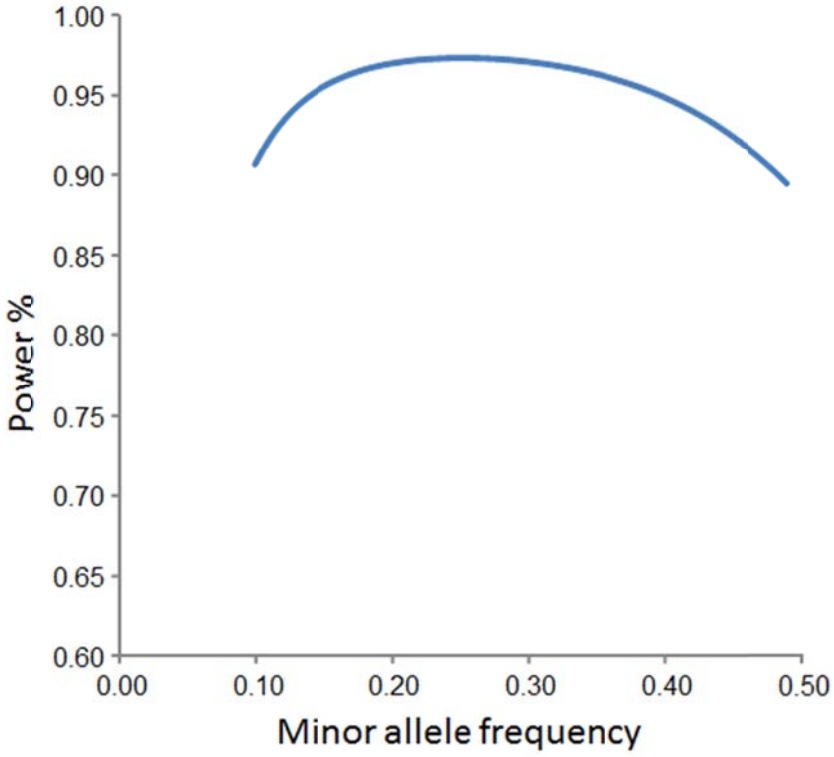

Figure S1. Power estimate for the case-control association analysis (assuming odds ratio value as 1.6; case, n = 527; control, n= 583). Statistical power was computed under the gene only hypothesis and the dominant model.

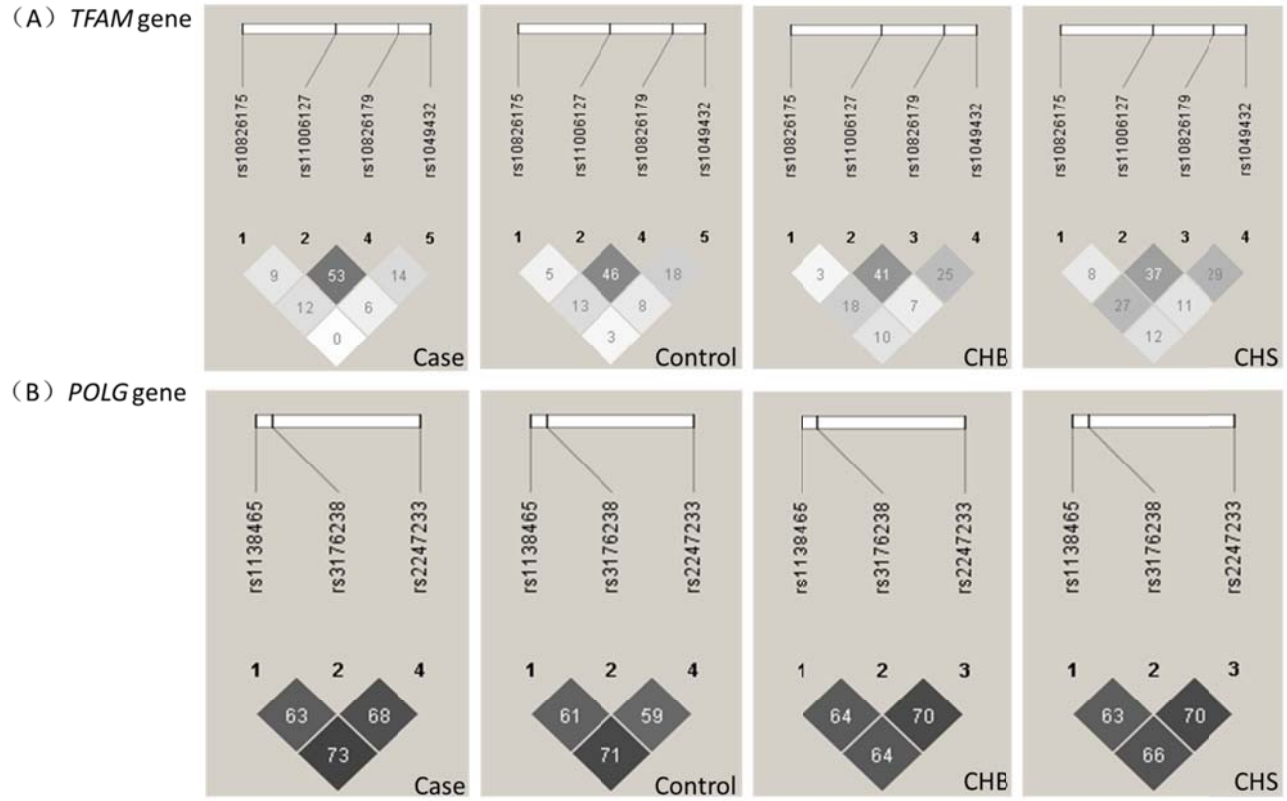

Figure S2. Linkage disequilibrium (LD) structures of *TFAM* (A) and *POLG* (B) in leprosy patients, healthy controls from Yuxi, and data of the corresponding SNPs in CHB and CHS from 1000 Genomes dataset [8]. Black squares represent high LD as measured by r^2, gradually coloring down to white squares of low LD. The individual square showed the r^2 value for each SNP pair (r^2 value is multiplied by 100).

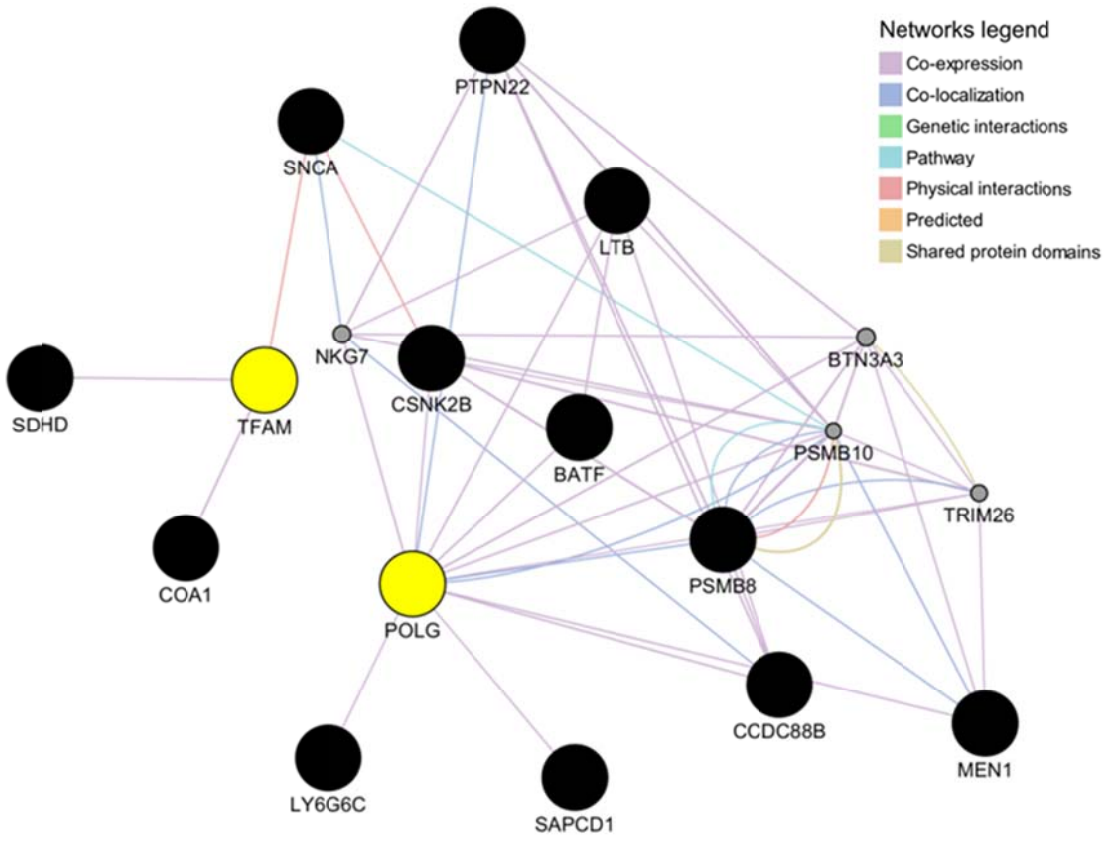

Figure S3. Protein interaction network of TFAM, POLG, and proteins encoded by the reported leprosy risk genes (Ref. [9] and references therein) using the GeneMANIA prediction server [10]. Only these proteins with a predicted interaction with TFAM and POLG were shown in the network.

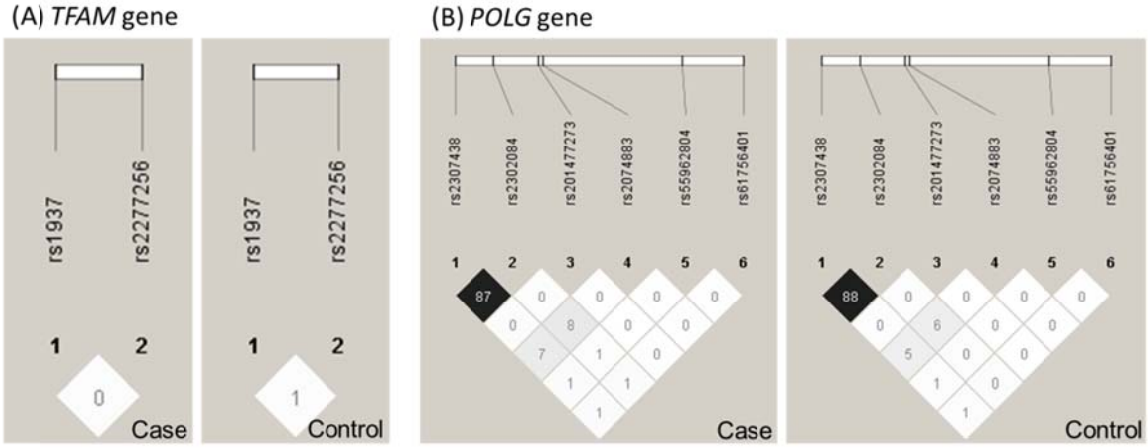

Figure S4. LD structure of variants with a MAF > 0.01 of *TFAM* (A) and *POLG* (B) in 798 leprosy patients and 990 healthy controls from the stage II sample (Wenshan population). A total of two variants (rs1937 and rs2277256) of *TFAM* and six variants (rs2307438, rs2302084, rs201477273, rs2074883, rs55962804 and rs61756401) of *POLG* were identified in the stage II sample. 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).

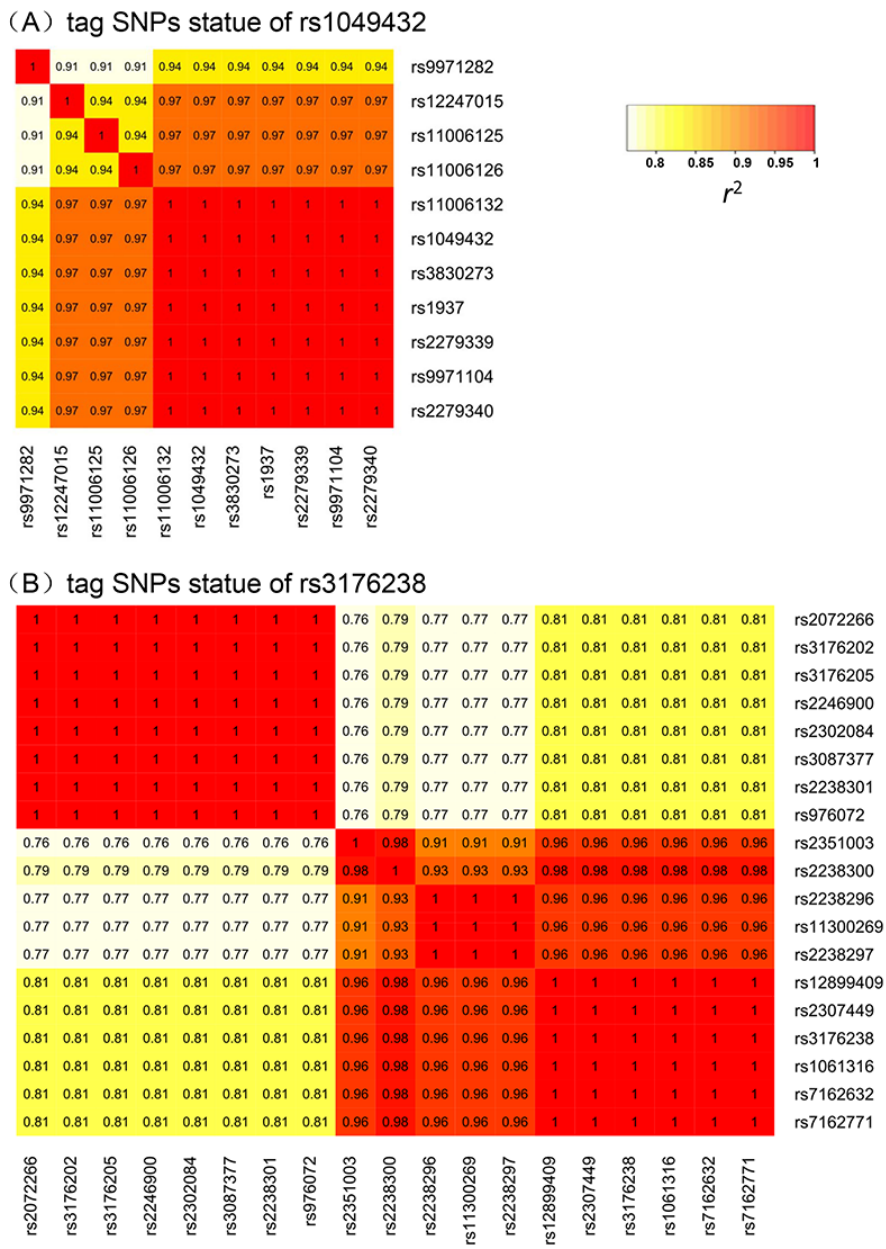

Figure S5. Linkage disequilibrium pattern of TFAM and POLG based on the CHB dataset from 1000 Genomes dataset (Ref.). The LD pattern of rs1049432 with other SNPs with a MAF > 0.01 in *TFAM* (A) and rs3176238 with other SNPs with a MAF > 0.01 in *POLG* (B) were shown here. Red squares represent high LD 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).

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